

Quality Assessment of Surface and Drinking Water of Nakla Paurosova, Sherpur, Bangladesh

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How to cite this paper: Sarker, S., Mahmud, S., Sultana, R., Biswas, R., Sarkar, P.P., Munayem, Md.A., Nur-E-Alam, Ali, Md.R., Wasim, M., Ali, Md.F., Faruque, Md.O. and Evamoni, F.Z. (2019) Quality Assessment of Surface and Drinking Water of Nakla Paurosova, Sherpur, Bangladesh. *Advances in Microbiology*, 9, 703-727.

<https://doi.org/10.4236/aim.2019.98043>

Received: May 30, 2019

Accepted: August 6, 2019

Published: August 9, 2019

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Abstract

Water is one of the most vital elements of ecosystem and human being, but unfortunately nowadays, the pollution of surface and drinking water is an alarming problem. The present work deals with the assessment of physico-chemical and bacteriological profile of several pond, jar and tube-well water samples to ensure its suitability for using and drinking. Total 30 samples were randomly selected and collected from Nakla Paurosova of Sherpur district by following the standard procedure. Bacteriological analysis was carried out by following the standard bacteriological methods. Most of the surface water sampling points were polluted by dumping of waste, cattle wash and were not suitable for drinking or other domestic purposes. Among three heavy metals, only Iron was detected in six tube-well water samples, one was also positive to arsenic, rest of the water bodies were negative to all of these metals. In case of most of the water bodies, different physicochemical properties were below standard limit. In pond water, the Total Viable Count (TVC) ranged from 2.7×10^7 cfu/100ml to 4.4×10^{15} cfu/100ml and Total Coliform Count (TCC) were 3.4×10^5 cfu/100ml to 4.8×10^{13} cfu/100ml, where the mean concentration of Heterotrophic Plate Count (HPC) was 2.4×10^5 cfu/100ml and 1.8×10^5 cfu/100ml in jar and tube-well water respectively. On the other hand, the Total Coliform Count of supply water was 33 cfu/100ml and tube-well water was 14 cfu/100ml. Fecal coliform was detected in all of the pond water samples, four jar and three tube-well water too. *E. coli* was present in all pond

water samples, and also detected in 80% supply and 50% tube-well water also. *Shigella* spp. was found in two pond water and in one supply water, where tube-well water was free from it. *Salmonella* spp. was also detected in 30% of pond and 20% of supply water, whereas absent in tube-well water. 50% of pond, 40% of supply and 30% of tube-well water were contaminated with *Vibrio* spp. The total counts of these pathogenic bacteria exceeded the acceptable limit both surface and drinking water and also showed resistance against a broad range of commercially available antibiotics. Survey-based result revealed that, peoples of the study area who were using or drinking these water, were suffering from various water borne diseases. These kinds of water sources pose a major threat to public health. So, public awareness, proper treatment and precise management are needed prior to use and drink of these water.

Keywords

Physiochemical Parameters, Bacterial Load, Antibiogram, Public Health, Nakla Paurosova

1. Introduction

Safe drinking water access for urban and rural populations in developing countries remains a challenge for sustainable development [1]. Water is essential to the existence of all living organisms. The environment, economic growth and developments of Bangladesh are all highly influenced by surface water because of its regional and seasonal availability [2]. This valued resource is increasingly being threatened as human populations grow. Demand also increasing day by day for more water of high quality for domestic purposes and economic activities [3]. Water quality can be defined by the chemical, physical and biological contents of water. The water quality of the surface water bodies of our country is decreasing by both conventional pollutants (heavy metals, pesticides), different organic or inorganic compounds and micro pollutants [4] [5]. Poor water quality may be caused by low water flow, municipal effluents and industrial discharges [6]. Degradation of water quality, depletion of water resources and loss of aquatic biodiversity are prominent features of the environmental landscape, which is requiring urgent attention at global and national scales [7]. Important physical and chemical parameters influencing the aquatic environment are temperature, rainfall, pH, salinity, dissolved oxygen and carbon dioxide. These parameters are the limiting factors for the survival of aquatic organisms. The physicochemical characteristics of the aquatic system have a direct influence on the types and distribution of aquatic biota [8]. Industrialization and excessive population lead to decrease the physicochemical properties of water due to disposal of human waste, industrial waste and a wide array of synthetic chemicals [9]. Heavy metals are also regarded as serious pollution of aquatic ecosystem because of their environmental persistence and toxicity effects on living organisms [10].

In the aquatic environment, the trace elements are partitioned among various environmental components such as water, suspended solids, sediments and biota [11]. The toxicity tests are necessary for water pollution evaluation because chemical and physical measurements alone are not sufficient to assess the potential effects on aquatic biota [12].

Water pollution by microorganisms is now a global concern. Pathogenic bacteria and the presence of antibiotic resistant bacteria in drinking water have become an emerging problem throughout the world [13]. In the disease-prone, humid, tropical region of Bangladesh, outbreaks of diarrhoeal diseases, often on an epidemic scale, are not unusual and the possible role of water-borne pathogens in these outbreaks has been emphasized due to polluted water with pathogenic microorganisms [14]. Among waterborne diseases of bacterial origin typhoid fever, bacillary dysentery and diarrhea are common in Bangladesh [15] [16]. Despite the availability and promotion of the use of safe water sources, water-related diseases remain an important cause of mortality and morbidity in Bangladesh [17]. The emergence of multidrug resistant and ESBL producing *E. coli* has been reported in numerous studies [18]. It becomes a warning barrier to infectious disease management in Bangladesh. The antibiotics resistant genes are either chromosome mediated or plasmid mediated and may be transferred by horizontal gene transfer [19]. Thus the dissemination of antibiotics resistance can be very frequent phenomenon if the resistant bacteria are present in the water. According to the World Health Organization (WHO), the mortality of water associated diseases exceeds 5 million people per year and from these more than 50% are microbial intestinal infections [20]. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Waste water discharges in fresh waters and costal seawaters are the major source of fecal microorganisms, including pathogens [21] [22]. The study was carried out to evaluate physicochemical and bacteriological profile of the several water bodies of Nakla Paurosova as well as antibiotic resistance pattern of the bacteria associated with these water bodies and to draw the awareness and concern of the consumers.

2. Materials and Method

2.1. Study Area and Sample Collection

Thirty water bodies (10 ponds, 10 tube-wells and 10 supply water samples) were randomly selected and collected from Nakla Paurosova, Sherpur district of Bangladesh. The study was carried out from July to November 2018. Approximately, 300 ml of water samples were collected in 500 ml of plastic bottles and before sample collection, all the plastic bottles were properly sterile by autoclave and cleaned by distilled water. Bottles were immersed below the water surface, filled, brought out of the water and properly closed. Then they were properly labeled with sample no, source, date, time etc. and samples were carried to the laboratory within six hours of collection (Figure 1).



Figure 1. Geographical location of study area.

2.2. Primary Data Collection

To investigate the alternation of physicochemical parameters and source of bacterial contamination of different water sources, literature based related primary data (such as-color, odor and surroundings of pond water bodies, deepness of tube-well, types of latrine and its distance from tube-well etc.) were collected with a semi-structured based questionnaire and further analysis [23].

2.3. Physicochemical Parameters Analysis

pH was determined by the digital pH meter (Hanna instruments). The Dissolved Oxygen (DO) was determined by digital DO meter (Model: D.46974; Taiwan). Digital Electrical Conductivity (EC) meter (Model: HM digital and made in Germany) was used to determine EC. Temperature was measured by the digital thermometer.

2.4. Detection of Heavy Metal

Arsenic, Lead and Iron were detected with Detection Kit (Hach Company, USA) and was carried out as the manufacturer's instruction.

2.5. Microbial Analysis

2.5.1. Total Viable Count (TVC)

For direct counting, spread plate technique was performed as described previously (APHA, 2003). Firstly, ten-fold serial dilution was carried out of every raw pond water samples. 0.1 ml of each sample is transferred by a micro pipette and spread on agar plates with a sterile bent glass rod. All the plates were inoculated at 37°C for 18 hours. Total count is expressed as colony forming unit per 100 ml (cfu/ml). Nutrient Agar media was used as culture media for enumeration of total viable bacteria in sample.

2.5.2. Heterotrophic Plate Count (HPC) of Drinking Water (Jar and Tube-Wells)

For the determination of heterotrophic plate count, 100 µl of serial tenfold dilution of jar and tube-well water samples were transferred and spread on Plate Count Agar (PCA) media using micro pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of plate count agar with a sterile glass spreader. One sterile glass spreader was used for each plate. The plates were then taken in an incubator at 37°C for 18 hours. After incubation at 37°C for 18 hours plates exhibiting 30 - 300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the heterotrophic plate count (HPC). The heterotrophic plate count was calculated according to International Organization for Standardization (ISO) (1995). The result of total bacterial count was expressed as the number of organism or colony forming units per 100 milliliter (cfu/100ml) of water samples.

2.5.3. Total Coliform Count (TCC)

Most probable number (MPN) test was done to identify the presence of coliforms in jar and tube-well water samples [24]. In presumptive MPN procedure, 15 lactose broth tubes were inoculated with the water samples. Five tubes received 10 ml of water, 5 tubes received 1 ml of water and 5 tubes received 0.1 ml of water. The number of tubes showing gas production and color change was compared to a standard table developed by American Public Health Association. The number of coliform was the MPN of coliforms per 100 ml of the water sample [25]. For pond water, 0.1 ml of ten-fold serial dilution of every sample was spread with a sterile glass spreader and incubated them at 37°C for 18 hours. After incubation the colony was counted by standard plate count method described as before.

2.5.4. 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 [26].

2.5.5. Isolation of Pathogenic Bacteria

For the identification of pathogenic bacteria, 100 µl of each sample were transferred into Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) media, Eosin methylene blue Agar (EMB) and Shigella Salmonella Agar (SS) media with ten-fold dilution. The diluted samples were spread as quickly on the surface of the plate with a sterile glass spreader and incubated at 37°C for overnight. The presence of pathogenic bacteria were observed and counted.

2.5.6. Cultural and Biochemical Examination of Samples

The cultural examination of water samples for bacteriological analysis was done according to the standard method by ICMSF [27]. The examination followed detail study of colony characteristics including the morphological and biochemical properties. In order to find out different types of microorganisms in water samples, different kinds of bacterial colonies were isolated in pure culture from the plate count agar (PCA), MacConkey agar (MCA), SS agar, TCBS agar and subsequently identified according to the methods described by Krieg [28]. Gram staining and biochemical reaction were performed for further confirmation of presumptively identified bacteria according to Bergey's Manual Determinative Bacteriology [29]. Among these 15 samples 12 kinds of biochemical test such as Kligler Iron Agar (KIA), Motility Indole Urease (MIU), Citrate, Voges Proskauer (VP), Oxidase, Catalase, Mannitol, Starch, Methyl Red (MR), Glucose, Lactose, Eosin Methylene Blue (EMB) were performed.

2.5.7. Antibiotic Sensitivity Test

Antibiotic susceptibility test was performed by disk diffusion method (Kirby-Baur method) using the commercially available antibiotic disk on Mullar-Hinton agar to assess the susceptibility and resistance pattern of the isolates [30]. 10 different types of common antibiotic disks (Himedia, India and Oxoid Ltd. England) were used in this study.

2.6. Evaluation of Public Health Impact

A field investigation and semi-structured questionnaire based survey was conducted among randomly selected 300 respondents (10 from each sampling sight, 50% male and 50% female) of the study area to determine the health status of people who were the consumers of these water.

2.7. Statistical Analysis

MS Excel 2013 was used for data analysis and presentation of graphs.

3. Results and Discussion

3.1. Analysis of Pond Surroundings

In Bangladesh, the major causes of contamination of surface water are dumping of domestic waste, cattle washing, connection with drains and opening of the latrine into water bodies etc. In this study, among ten ponds, only two (pond-4

and pond-7) were connected with drains, four (pond-2, pond-4, pond-7 and pond-8) were polluted directly by dumping of waste, 50% (pond-1, pond-2, pond-5, pond-7 and pond-9) was used for washing of cattle by the people around it and 20% (pond-4 and pond-8) was contaminated with direct fecal coliforms by the opening of latrines into these water bodies (**Figure 2**).

Due to dumping of domestic, untreated industrial and municipal wastes, the majority numbers of water reservoirs in Bangladesh are becoming hazardous for human, animal and aquatic lives [31]. On the other hand, cattle washing on ponds degrade water quality and sanctions concern microbial contaminants which are threatening to human health and ecological life [32].

3.2. Surrounding Environment of Tube-Wells

The microbial quality of tube-wells water depends on the deepness of tube-wells, its distance from latrine and the latrine's condition. Here, the deepness of 10 tube-wells were varies from 60 to 105 feet and the mean deepness was 85.5 feet. The distance from latrine to tube-wells was counted about 9 to 36 feet and the mean distance was about 20 feet. Three types of latrine were found by considering latrine condition; four were direct pit, three were offset and three were soak with septic tank (SWST) (**Figure 3**). In previous studies it was found that, low deepness of tube wells and less distance from latrine indirectly related to the microbial contamination of the water of these sources [33].

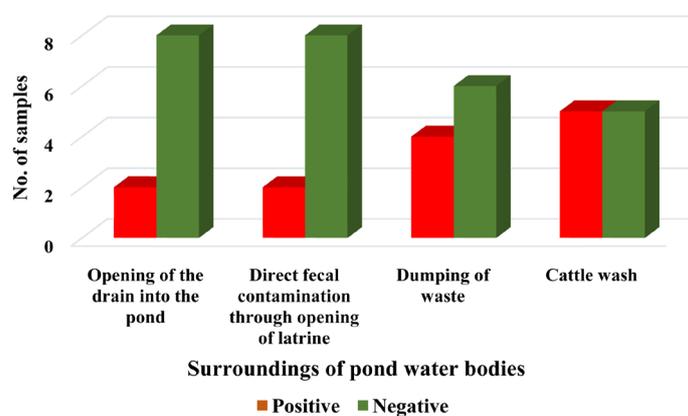


Figure 2. Surroundings of pond water.

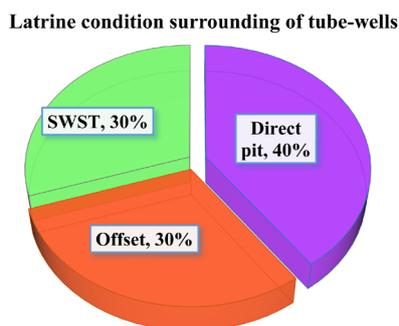


Figure 3. Surrounding of tube-wells.

3.3. Physiochemical Parameters Analysis

3.3.1. Color and Odor of Pond Water

The color and odor of pond water were observed visually because it is an important premier indicator for investigating water quality which indicates its suitability for survival of fishes or other aquatic creatures, and for domestic works. According to the guidelines of WHO (2004) for water quality, the color level below 15 true color units (TCU) are acceptable for most aquatic inhabitants and above 15 TCU are detected by naked eyes [34]. In this study, about 30% of pond were appeared turbid (pond-4, pond-7 and pond-8) where, the other 30% (pond-1, pond-5, and pond-9) were light green, rest of them were deep green (20%) and brown (20%) in color (Figure 4). The odor of water is directly proportional to the level of contamination. Among 10 samples, about 60% were smelling normal and 40% having bad odor (Figure 5). Bad odor can be resulted from over bacterial growth along with discharging of domestic and industrial waste from the surrounding area [35].

Colors of pond water

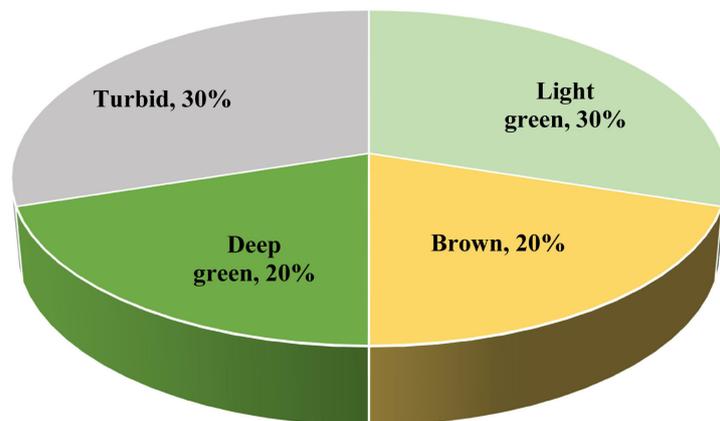


Figure 4. Colors of pond water samples.

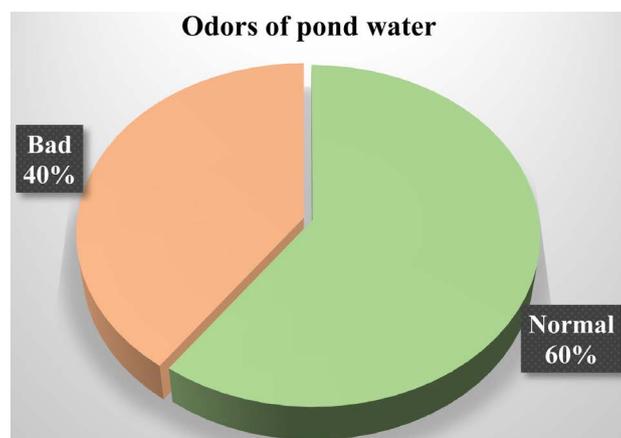


Figure 5. Odors of pond water.

3.3.2. Temperature of Pond Water

The variation of water temperature generally depends on season, geographic location, sampling time and temperature of effluents [36]. The temperature of ten pond water bodies was founded ranging from 27.8°C to 29.5°C, and the mean was 28.6°C. In another similar study conducted in Tangail area, recorded the temperature of ponds water range from 17.9°C to 23.0°C and the average value was 20.7°C [37]. Both of these results are closely related to the optimum temperature (20°C - 30°C) that favorable for living of aquatic habitants [38].

3.3.3. The pH of Water Bodies

pH is one of the most important attribute of any aquatic system and it can be changed due to alteration of physicochemical and environmental factors of water [39] [40]. The mean pH of pond, jar and tube-well water was recorded as 6.96, 6.93 and 7.1 respectively. The highest pH was 7.55 (Pond-3), 7.55 (Jar-6), 7.75 (TW-3) and lowest was 6.37 (Pond-8), 6.26 (Jar-7), 6.69 (TW-9) for pond, jar and tube-well water in some respects (Figure 6). According to WHO's standard, an appropriate range of pH for drinking water is 6.5 - 8.5 [41]. The average pH of jar and tube-well water of the study area was within the standard limit. Some previous works reported the mean pH of supply water was 6.84 in Sherpur, Bangladesh [33] where the average pH of drinking water was 6.27 at rural area of Bangladesh [42]. On the other hand, a study on pond water quality of Tangail area found the average pH was 7.68 [37]. pH range 7.5 to 8.4 is good for the growth of algae, 6.0 to 7.2 is optimum for laying fish [43], 6 to 8 is good for growth and reproduction of fish species, but pH below 5 or as high as 9 to 11 do not allow fishes to reproduce, and also cause slow growth [44].

This current work recorded the pH of ponds water as slightly alkaline, which may due to inorganic sediments or domestic and agricultural wastes disposed in these water bodies.

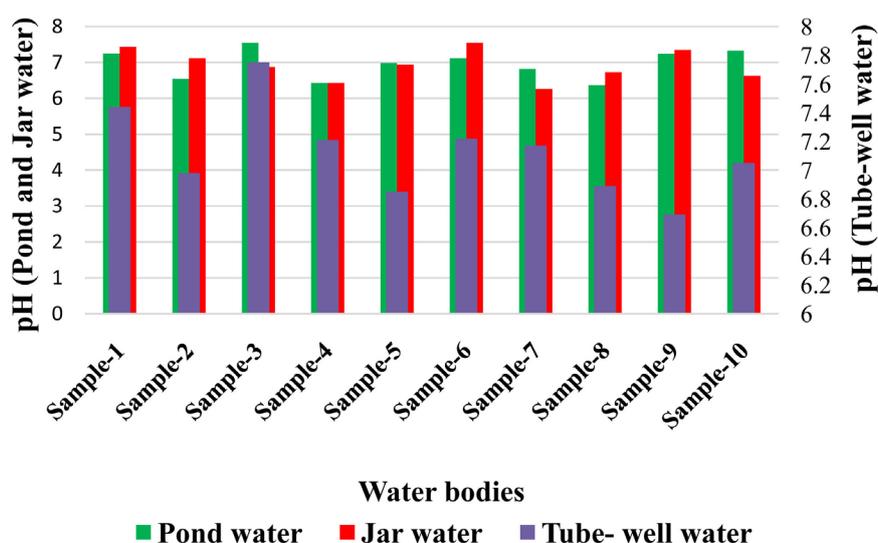


Figure 6. pH of all water bodies.

3.3.4. Dissolved Oxygen (DO) of Water Bodies

Dissolved oxygen is the only sources of oxygen for respiration of aquatic creature. Insufficient and imbalance DO may lead to cause of sudden death of these creature. The average DO of pond, jar and tube-well water was determined as 4.3, 3.7 and 5.8 mg/l. The maximum concentration of DO was 5.4 mg/l (pond-6), 4.3 mg/l (jar-6), 7.1 mg/l (TW-3) and the minimum was 3.4 mg/l (pond-8), 3.1 mg/l (jar-3), 4.9 mg/l (TW-9) for pond, jar and tube-well water relatively (Figure 7). Department of Environment, Ministry of Environment and Forest, Government Republic of Bangladesh set the standard value of DO for drinking water for Bangladesh as 6 mg/l or more [45]. Previous studies found the average DO of pond water was 5.23 mg/l in Tangail area [37] and 7.7 mg/l in Thakurgaon Sadar Upazila [46]. The mean DO contents of supply water and average DO of tube-well water in Fulbaria, Bangladesh were reported as 3.1 mg/l and 3.1 mg/l respectively [33]. The average DO of water bodies in study area was below the satisfactory level, so why pond water was not suitable for fish production and aquatic eco-system where tube-wells or tap water for drinking. Due to the pollution of the surface water, lack of proper treatment or excessive water treatment for purification, and the over growth of microorganisms in water bodies may be the possible causes of low level of DO.

3.3.5. Electrical Conductivity (EC)

Electrical conductivity (EC) is a quantification of the capability of a liquid to conduct an electric current. Water shows important conductivity when inorganic cations and anions (such as sodium, calcium, magnesium, aluminum, chloride, nitrate, sulfate, phosphate etc.) were dissolved [47]. The highest and lowest EC was 341 $\mu\text{S}/\text{cm}$ (pond-7) and 196 $\mu\text{S}/\text{cm}$ (pond-10), and the average was 266.3 $\mu\text{S}/\text{cm}$ in pond water.

The maximum and minimum EC was 610 $\mu\text{S}/\text{cm}$ (Jar 6) and 417 $\mu\text{S}/\text{cm}$ (Jar 9) in jar water and the average was 495.2 $\mu\text{S}/\text{cm}$. On the other hand, the highest and lowest EC of tube-well water was 965 $\mu\text{S}/\text{cm}$ (TW 6) and 635 $\mu\text{S}/\text{cm}$ (TW 5)

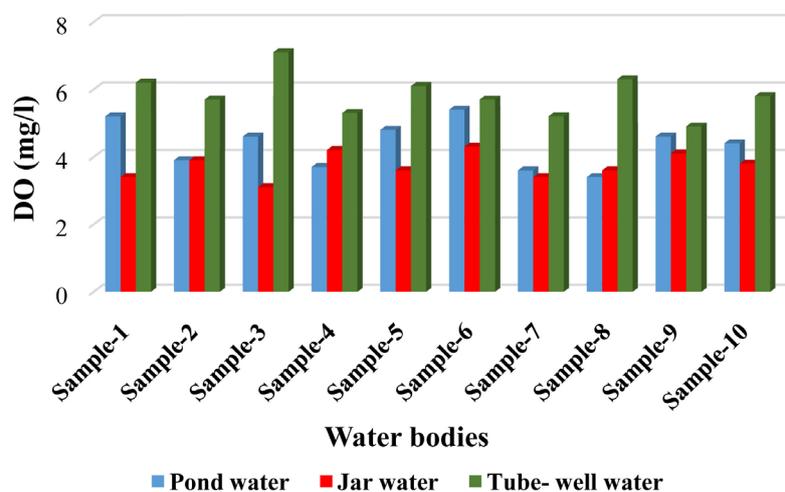


Figure 7. Values of DO of all water bodies.

where the average was 760.5 $\mu\text{S}/\text{cm}$ (Figure 8). The average EC of pond water of Thakurgaon Sadar Upazila was reported as 145 $\mu\text{S}/\text{cm}$ [46] and water with an EC value of 0 - 750 $\mu\text{S}/\text{cm}$ is considered as excellent for irrigation [48]. A study reported the EC value of the bottled waters of Dhaka city, Bangladesh was 38.4 to 493.0 $\mu\text{S}/\text{cm}$ and the mean was 173.3 $\mu\text{S}/\text{cm}$ [49]. The maximum permissible limit of EC in Bangladesh is 1200 $\mu\text{S}/\text{cm}$ for safe drinking water [45].

3.4. Presence of Heavy Metals

The presence of heavy metal in drinking water may lead to complications of kidney, and in pond water may cause ionic imbalance. None of these three heavy metals, Arsenic (As), Lead (Pb) and Iron (Fe) were detected in both pond and jar water, but iron was detected in six samples in tube-wells where only one was positive to arsenic (Figure 9). But Lead (Pb) and Iron (Fe) were detected in pond water by another study conducted in Ashulia area, Bangladesh [50]. A recent study showed that arsenic was not detected in jar water of Dhaka city but iron was in acceptable limit [49]. Most of the tube-well water of Kushtia district, Bangladesh were positive to arsenic and iron, but majority of them were within reasonable limit [51].

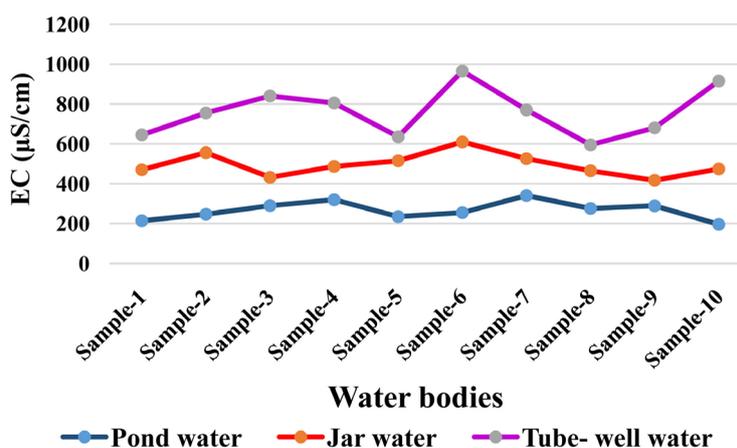


Figure 8. Electrical conductivity of water bodies.

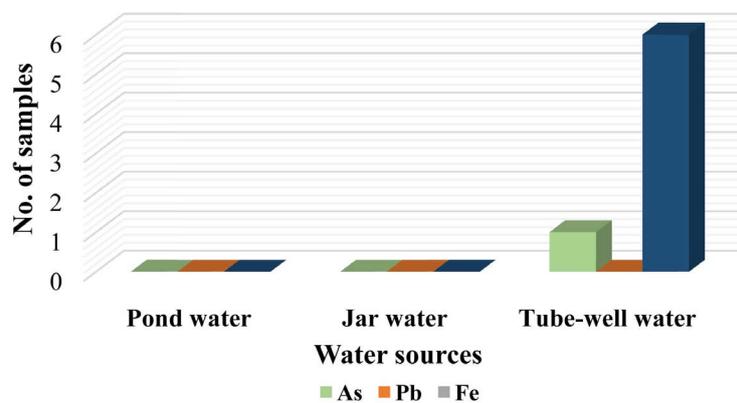


Figure 9. Presence of heavy metals in water bodies.

3.5. Microbiological Quality Analysis

3.5.1. Total Viable Count (TVC) of Pond Water

Total viable count (TVC) indicates a quantitative estimate of the concentration of microorganisms in a sample. Among ten pond water samples, the highest TVC count was 4.4×10^{15} cfu/100ml (pond-8) and the lowest count was 2.7×10^7 cfu/100ml (pond-3) (Figure 10). A previous study reported that the average TVC of pond water in Dhaka university campus was 2.7×10^6 cfu/100ml [52]. Surface water is the source of a variety of microorganisms for its surrounding environment. Due to the connection of drain and latrine in pond water and dumping of different contaminated wastes may be the prime cause of these contaminants.

3.5.2. Heterotrophic Plate Count (HPC) of Drinking Water

Heterotrophic plate count (HPC) is a method that measures colony formation on culture media of heterotrophic bacteria of a particular sample. It is the measurement of bacteriological quality of drinking water in public, semi-public and private water supply systems. In case of jar water, the highest HPC count was 3.1×10^5 cfu/100ml (jar-4) and the lowest count was 1.8×10^5 cfu/100ml (jar-10). The mean HPC of jar water was 2.4×10^5 cfu/100ml. But in tube-well water the count was 2.6×10^5 cfu/100ml to 1.3×10^5 cfu/100ml and the average count was 1.8×10^5 cfu/100ml (Figure 11). The average HPC of tap water of Jamalpur district and tube-well water in Tangail district was reported as 4.2×10^7 cfu/100ml and 2.7×10^7 cfu/100ml respectively [53]. According to WHO guideline, the values of HPC should remain within 1.0×10^3 cfu/100ml for drinking water but all determined values were above than the standard value [42]. Lack of appropriate treatment of supply water, unhygienic bottle condition, poor sanitation condition and leakage of pipes of tube-wells may be the leading causes of contamination of drinking water in the study area.

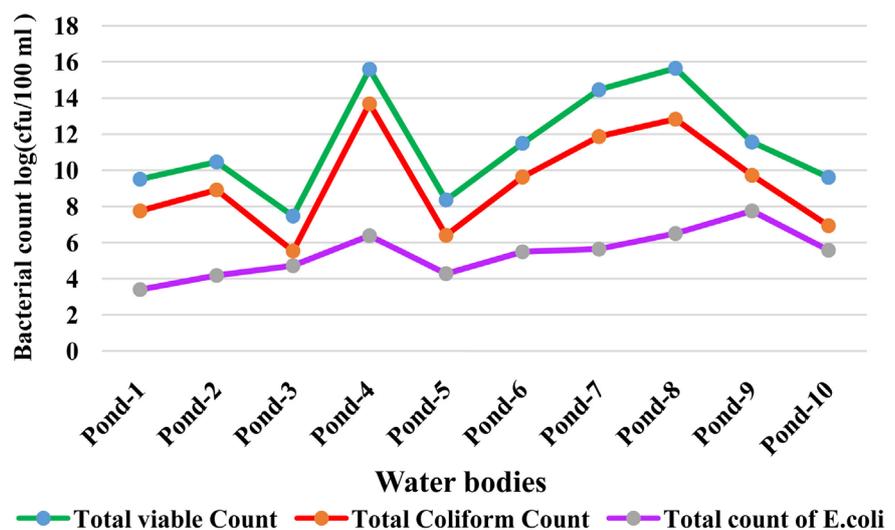


Figure 10. TVC, TCC and total *E. coli* count of pond water.

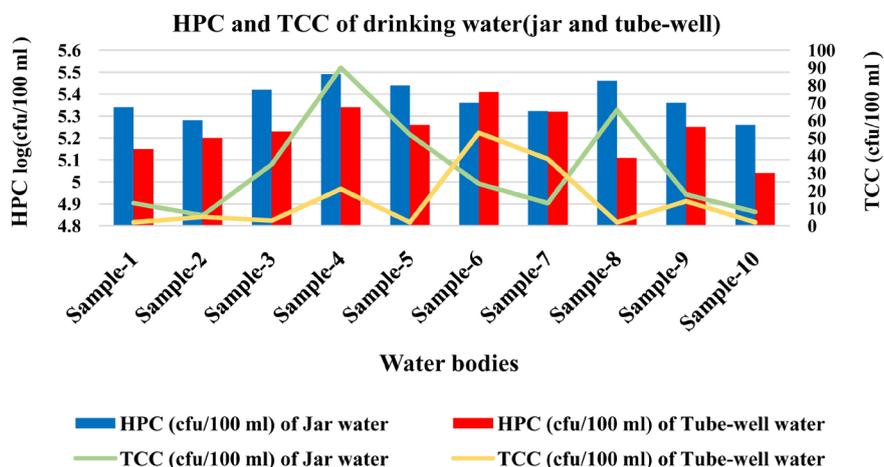


Figure 11. HPC and TCC of drinking water.

3.5.3. Total Coliform Count

Coliform bacteria is a group of pathogenic bacterial organisms which may present in food, water, soil and aquatic environment. Total coliform count used as an indicator to determine the microbial quality of treated water and its suitability for human consumption or not. The presence of excessive coliform bacteria suggests that disease causing pathogenic bacteria or fecal coliform may contaminate water sample [54]. In pond water, the lowest value of TCC was counted as 3.4×10^5 cfu/100ml (Pond-3) and the highest was 4.8×10^{13} cfu/100ml (Pond-4) where the mean value was 2.5×10^6 cfu/100ml (Figure 10). In a previous study, the average values of TCC was 4.5×10^{18} cfu/100ml in different surface water of Dhaka, Bangladesh [55] and many other studies also support the similar result regarding TCC [52] [56] [57]. In jar water, the highest number of coliform was 90 (Jar-6), lowest was 6 (Jar-2) and the average was about 33 (Figure 11). A past study reported that, the range of total coliform count was 33 cfu/ml to 1.0×10^3 cfu/ml in house tap water at Dhaka, Bangladesh [58]. In tube well water, the lowest number of coliform was counted as ≤ 2 (TW-1, 5, 10) and the highest number was 53 (TW-6) where the mean was about 14 (Figure 11). A previous study on tube-well water of Magura district in Bangladesh reported that all underground water sources contained total coliforms ranging from ≤ 2 cfu/100ml to ≥ 100 cfu/100ml [59]. Connection of the drains with pond, opening of the latrine on it or directly fecal contamination by different faces etc. are the most probable vital causes for the presence of coliform in pond water.

On the other hand, coliform contamination of tube-well water may occurred due to less distance of latrine from tube-well, low deepness of tube-well, latrine's surrounding condition, personal unhygiene, cross-contamination or pre-existing microbial contaminants present in handle or upper surface of tube-well.

3.5.4. Presence of Fecal Coliform

Fecal coliform is a large sub-group of coliform bacteria that exist in faeces. Certain types of fecal coliforms can be found naturally on plants and soils [55]. Fecal coliforms may detect in drinking water due to the contamination of sewage or

other sources of fecal matter [54]. It may cause severe gastrointestinal illness, however their presence in drinking water may indicate the presence of disease-causing organisms [60]. In this study, fecal coliform were detected in all ponds water, 4 jar water (out of 10) and 3 tube-well water samples (out of 10) (Figure 12). A study reported the similar result of pond water at Dhaka, Bangladesh [61] where another study found that only 18.8% tube-well water and 16.6% of supplied water samples were free of fecal coliform [62]. Presence of fecal coliform in these sources indicate inappropriate hygienic practice, inadequate proper sanitation system and lack of appropriate fecal management, which are now becoming matter of concern and damning news for public health.

3.5.5. Biochemical Test for Presumptive Conformation of Bacterial Species

Four types of distinct bacterial species (*E. coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp.) were isolated and identified by observing different morphological characteristics on selective media and further confirmed with standard short Biochemical test (Table 1).

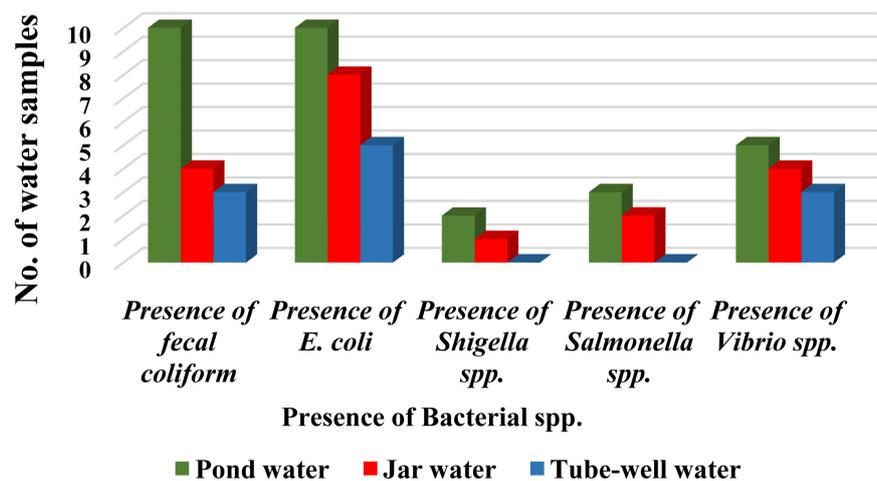


Figure 12. Presence of pathogenic bacteria.

Table 1. Reaction of biochemical test.

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	-	+	AG	+	<i>E. coli</i>
-Ve	-	K	A	G	+	+	-	+	-	+	+	+	+	+	+	-	<i>Vibrio</i> spp.
-Ve	-	K	A	G	+	-	+	+	-	-	-	A	-	+	-	-	<i>Salmonella</i> spp.
-Ve	-	K	A	-	-	-	-	-	-	-	+	-	-	+	+	-	<i>Shigella</i> spp.

K = Alkaline, A = Acid, G = Gas, AG = Acid and Gas, + = Presence, - = Absence.

3.5.6. *Escherichia coli* Count

Presence of *E. coli* in water is a common phenomenon but a high count of *E. coli* indicates that the water is unfit for drinking and any kinds of household works. High number of *E. coli* also indicates probable recent fecal contamination and there is a greater risk that pathogenic *E. coli* may present [63]. *E. coli* causes severe gastrointestinal-tract related complications like diarrhea, dysentery, urinary tract infections, pneumonia and even meningitis [64]. There is a clear relationship between the concentration of *E. coli* in a particular water sample and the possibility of onset gastrointestinal complications in human that exposed to the water through drinking [65]. This study has exposed the presence of *E. coli* in pond water where the maximum concentration was 5.7×10^7 cfu/100ml (Pond-9) and the minimum was 1.5×10^4 cfu/100ml (Pond-2). The mean concentration of *E. coli* was 6.4×10^6 cfu/100ml (Figure 11) and it exceeded the EPA's recommended limit [60]. A study reported that the average concentration of *E. coli* at pond water of Dhaka city was 2.12×10^2 cfu/100ml and also found the presence of *E. coli* at household water [66]. In our study area, 80% of jar and 50% of tube-well water samples were contaminated with *E. coli*. Another study reported that, about 40% of tube-well water of Noakhali district, Bangladesh contaminated with *E. coli* [67].

3.5.7. Presence of *Shigella* Spp.

Shigella spp. is an inhabitant of the intestinal tract of human and other primates [68]. It is typically spread through fecal-contaminated drinking water, food or by direct contact with an infected person. In water, *Shigella* spp. is able to survive a few days at room temperature and this survival reason favors the transmission through water [69]. A large number of waterborne outbreaks have been occurred because of shigellosis over the past years [70]. Among 30 water bodies of the study area, 20% of pond and 10% of jar water samples were contaminated with *Shigella* spp. and tube-well water was free from this organism (Figure 12). *Shigella* spp. can survive transit through the stomach since they are less susceptible to acid than other bacteria; for this reason, as few as 10 to 100 organisms can cause disease [71]. Jar with contaminants and cross contamination during personal handling can be considerable reason for contamination of jar water. Open latrine and drainages were the major source of fecal contamination of pond water that can break out serious shigellosis among this water consumer.

3.5.8. Presence of *Salmonella* Spp.

Salmonella spp. cannot multiply significantly in the natural environment, but they can survive several weeks in nutrients rich water and soil with favorable temperature, humidity, and pH [72]. The intestinal tract of human and animals are the major habitat of *Salmonella* spp. that causes four clinical manifestations: gastroenteritis, bacteremia, typhoid fever, and carrier state in persons who had been previously infected [73]. Here, 30% of pond and 20% of jar water were contaminated with *Salmonella* spp. tube-well water were free from *Salmonella* spp. (Figure 12) that is similar with another study [74]. Microbiological study

on supply water in Dhaka city reported that, 50% of supplied water were contaminated with *Salmonella* spp. [75].

3.5.9. *Vibrio* Spp. Count

Vibrio is a genus of ubiquitous bacteria found in a wide variety of aquatic and marine habitats [76]. *Vibrio cholerae* caused cholera which is a major public health problem in developing countries like Bangladesh. It is a waterborne pathogen and transmitted through aquatic sources or the feces of an infected person [77]. Several *Vibrio* species can infect human and *V. cholerae* is the most pathogenic among of these species [78] [79]. *Vibrio* spp. was detected in 50% of pond water in this study where their count was 1.2×10^3 to 5.7×10^4 cfu/100ml, and the average was 1.3×10^4 cfu/100ml. The presence of *Vibrio* spp. was detected in 40% of jar and 30% of tube-well water samples (Figure 12). A study reported that about 58% pond water were contaminated with *Vibrio* spp. in Khulna Division, Bangladesh [80]. In another case, about 27% *Vibrio* spp. were isolated in Dhaka city and 25% of tube-well water were contaminated with it at coastal area, Bangladesh [81] [82]. Contamination of tube-well water appears related to a number of factors, including proximity of latrines or drains to the tube wells, tube well depth or method of completion.

3.6. Antibiotic Resistance Pattern

A great deal of health threat may arise through the spreading of the antibiotic resistance to normal flora or creating the environment which would be favorable for opportunistic pathogens. Among the isolated organisms, *E. coli* was highly resistance to Ampicillin (70%), Amoxicillin (70%) and showed 100% sensitivity to Ciprofloxacin, 80% to Norfloxacin, 70% to both Streptomycin and Azithromycin, and 60% to Tetracycline and Erythromycin as well (Figure 13).

Shigella spp. was highly resistance to Ampicillin (90%) and highly sensitive to Ciprofloxacin (70%) (Figure 14) where *Salmonella* spp. was 100% resistance to Ampicillin and highly sensitive to Ciprofloxacin (60%) (Figure 15). On the other hand, *Vibrio* spp. was highly resistance to Amoxicillin (60%) and highly sensitive to Ciprofloxacin (60%) (Figure 16). Some previous studies reported that

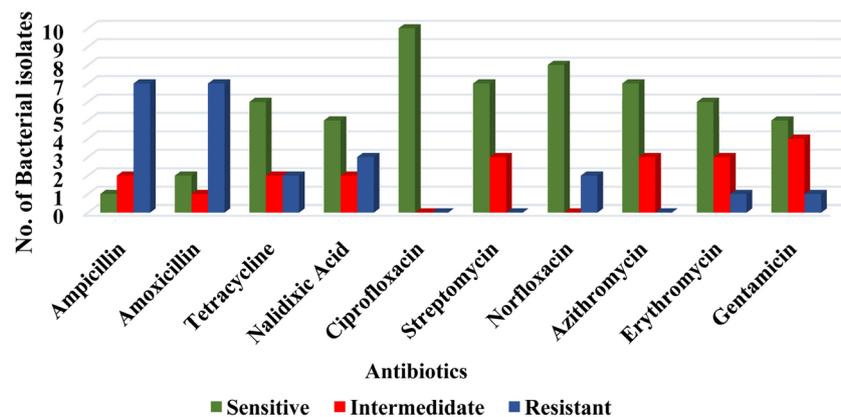


Figure 13. Antibiotic resistance pattern of *E. coli*.

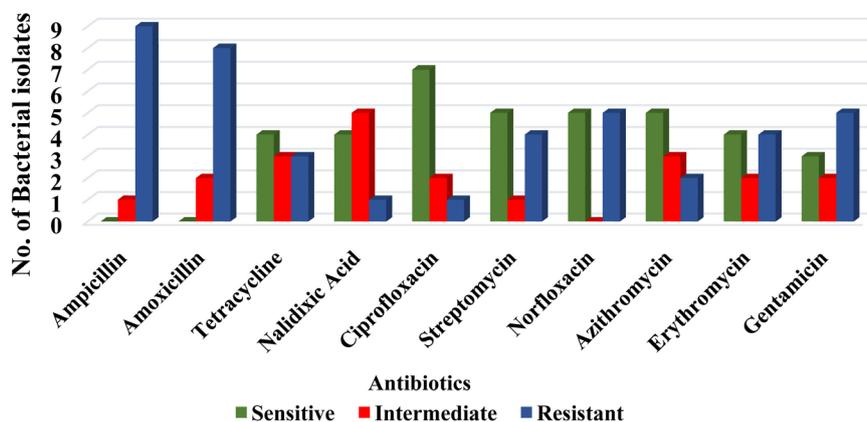


Figure 14. Antibiotic resistance pattern of *Shigella* spp.

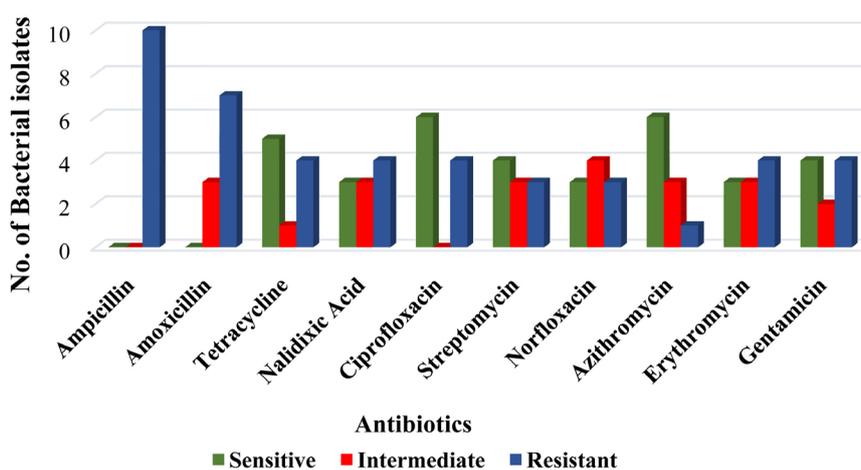


Figure 15. Antibiotic resistance pattern of *Salmonella* spp.

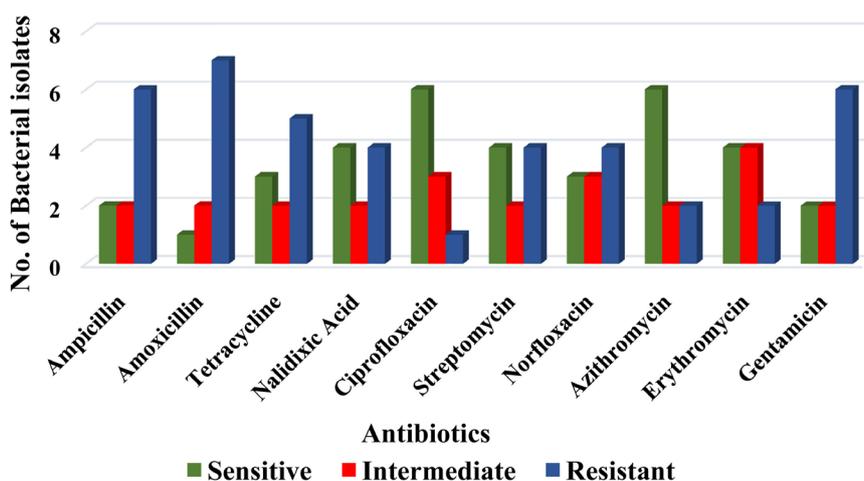


Figure 16. Antibiotic resistance pattern of *Vibrio* spp.

different bacteria that were isolated from the pond, tube-well and supply water were also resistant to some common commercially available antibiotics [83] [84] [85].

This multi-drug resistance might have arisen due to misuse of antibiotics or prolonged use of single antibiotic, horizontal gene transfer from pathogenic to common nonpathogenic bacteria present in water bodies, insufficient or lack of accurate management of hospital and medical wastes etc. The result suggested that immediate precautions should be taken by the government to stop the selling of antibiotics without prescription, should be increase the public awareness and will be ensure the proper use of antibiotics with perfect dosages by doctors and patient, respectively.

3.7. Prevalence of Waterborne Diseases at the Study Area

Water contamination is one of the main causes of health problems in human being. About 2.3 billion people are suffering from water related diseases worldwide [86] and more than 2.2 million people die in developing countries every year due to waterborne diseases [87]. Waterborne disease is the major burden in the study area. Most of these water borne diseases are attributed to the contaminated water that contains various infectious pathogens. Further, few people have been found in the study area who use proper water purification systems are free from these waterborne diseases. To investigate the impact of contaminated water on public health, 10 persons were taken from each sampling site during the study period of samples collection and total respondents were 300 (50% were male and 50% female). Among the respondents, most of the people were aware about the reasons and treatment of scabies (65%), skin diseases (69%) and diarrhea (54%), and very few were conscious about campylo-bacteriosis (1%), salmonellosis (2%), botulism (7%) and hepatitis (23%) (Table 2). Here, it was noticed that few people were suffering from those diseases from long time.

Table 2. Impact of waterborne diseases on public health.

Diseases	Clear concept about reason and treatment	Suffering from a long time	Frequently	Affected within last year	Affected within last month	Affected within last week	Took antibiotic
Diarrhea	170	7	12	35	6	8	28
Dysentery	140	3	8	5	16	5	23
Cholera	95	-	-	9	8	-	15
Typhoid	112	-	-	8	2	4	14
Hepatitis	68	-	-	5	3	1	6
Botulism	22	-	13	11	8	2	25
Campylo-bacteriosis	2	-	-	-	-	-	-
Salmonellosis	7	-	-	5	-	-	5
Scabies	198	14	7	32	4	9	2
Skin diseases	205	19	26	3	8	2	4
Skin cancer	78	-	-	-	-	-	-
Dad	134	5	-	2	3	-	-
Itching and rash on the skin	163	22	18	9	3	7	5

Highest rate for long time suffering of diseases was found for itching and rash on the skin (7%). From 13 common diseases, most frequently occur disease was diarrhea. More than 11% people were found to be affected last year by diarrhea and scabies (10%) and 5% people were affected last month by dysentery. 3% people were affected by scabies within last week. Among the diseases, highest number of antibiotic was taken for diarrhea (9%), botulism (8%) and dysentery (7%). From this study a clear scenario that the dose of antibiotics were not completed by most of the respondents and the efficiency of these antibiotics were not satisfactory that also indicates the occurrence of antibiotic resistance. Government authorities should establish protocols to monitor the occurrence of water borne diseases and develop awareness programs to inform the communities about the symptoms, treatment and pattern of taking antibiotics.

4. Conclusion

Water is vital for the daily activities of life for drinking and different household works which is continuously polluted by municipal wastes and contaminated with different pathogenic bacteria. The quality of this water may impact on the incidence of various water-borne infectious diseases of human and threat for aquatic biota. The present study revealed that the physiochemical and bacterial quality of the water samples was out of the standard limit of WHO and risky for drinking, aquatic lives, domestic purposes due to the presence of pathogenic bacteria. The antibiotic resistant bacteria in these water resources pose a serious public health threat and responsible genes for antibiotic resistance are transferring from pathogenic to non-pathogenic organisms. It can be recommended that the government authorities should establish protocols to monitor water quality and develop awareness programs to inform the communities about the status of the water quality to reduce the burden from water-borne infection diseases.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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