ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* AND *SALMONELLA* FROM DRINKING WATER IN AND AROUND THE SAU CAMPUS

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TO

MY BELOVED PARENTS

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ABSTRACT

This study was conducted to investigate the *E. coli* and *Salmonella* from drinking water in SAU campus and its surrounding areas from January to August 2021. A total of 60 samples were collected from different halls, faculties and neighboring market areas of the SAU campus. Bacteria was identified observing the growth properties in different media, staining properties, microscopic observation and biochemical test. The characteristics colonies of *E. coli* were red to bright pink colored in MA agar & greenish red colored with faint metallic sheen in EMB agar. The characteristics colonies of *Salmonella* were black colored in SS agar & gray colored in EMB agar. Both bacteria observed in rod shaped, gram negative, single or paired arranged under microscope. In biochemical test both bacteria showed Catalase test, MR test positive and VP test negative. In case of Indole test *E. coli* showed positive but *Salmonella* showed negative indication. The higher prevalence (66.67%) was in Krishokrotno Sheikh Hasina Hall & Sheikh Sayera Khatun Hall. For faculties, the lower (33.33%) prevalence was in Sheikh Kamal Bhaban and higher (66.67%) was in Agriculture Faculty Bhaban. All samples collected from college gate street food shop were positive. The prevalence of *E. coli* & *Salmonella* in this study areas were 20% and 33%. The mixed contamination was 5%. So, direct consumption of water should not safe for us. It can cause serious health hazard.

Keywords: *E. coli*, *Salmonella*, Drinking Water, SAU Campus, Prevalence

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LISTS OF ABBREVIATIONS

SAU	Sher-e-Bangla Agricultural University
spp.	Species
E. coli	*E. coli*
NA	Nutrient Agar
SS	*Salmonella*-Shigella
EMB	Eosin Methylene Blue
MAC	MacConkey Agar Media
MR	Methyl-Red
VP	Voges Proskauer
Gm	Gram
Cfu/ml	Colony forming unit per milliliter
NB	Nutrient broth
%	Percentage
+ve	Positive
-ve	Negative
Gm+ve	Gram positive
Gm-ve	Gram negative

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Water is the most needed element of our life. water safety is always very important for healthy lifestyle but many people can't get clean and safe water for drinking and household purposes (Acharjee *et al*., 2013). Water contamination for drinking purpose has been a serious issue in all over the world. Bangladesh is a developing country, here sanitation & hygiene aren't maintained properly. The contamination of drinking water become alarming due to the spread of many more serious diseases which is a major cause of death throughout the country (Majumder *et al*., 2011; Uddin., 2018).

Diseases due to intake of contaminated drinking water causes a principal issue on human & animal health. To improve the quality of this by identifying the causes of contamination can introduce significant benefit to the human and animal health. Supply of contamination free, pure drinking water can improve health, productivity & livelihoods. Poverty reduction & sustainable development can be accomplished by the accessibility and availability of fresh & clean drinking water (Tekpor *et al*., 2017).

Around 5 million death cases occur due to intake of contaminated drinking water and lack practice of hygiene and among this death cases 50% caused by the Cholera. Typhoid, Fever, Dysentery, Cholera etc. are common diseases reported in Bangladesh those are transmitted frequently through contaminated drinking water & that is also a major public health concern. Water can be contaminated by these following pathogen- *E. coli* (*Escherichia coli*), *Salmonella spp*., *Shigella spp*., *Streptococcus spp*., *Vibrio spp*., *Bacillus spp*., *Pseudomonas spp*. etc. Those are the bacteria which are responsible for the disease like- enteric fever, diarrhea, typhoid, dysentery and many other illnesses (Munshi *et al*., 2012) & those are reported by patients frequently from various region of Bangladesh (Majumder *et al*., 2011). The river water is also a source of transmission for enteric diseases (Reshu *et al*., 2015)

If any disinfection procedure is applied in supply systems of drinking water & the disinfection procedure can be failed. The effect of failure of disinfection procedure can cause serious health hazards through this contamination. By improving only, the quality of source water doesn't always decrease the incidence of diarrhoeal disease (Brisco., 1978), because the pathogens of diarrheal diseases can also be transmitted by foods or

by using contaminated water for other household uses, e.g., consumption of raw vegetables, washing utensils etc. Municipal piped water in many developing countries is not safe because improper maintenance of pipes, lack of pressure, less chlorination etc. *Vibrio cholerae* is a pathogen for causing several diarrheal diseases, it was frequently isolated from municipal water supply systems which was non-chlorinated & associated with large number of cholera epidemics in Peru (Ries *et al*., 1992). Even drinking unboiled municipal water remained a primary cholera infection source in Ecuador (Weber *et al*., 1994). In case of Indian sub- continent quality of river water considered as potential source of Fecal coliform bacteria (Shukla *et al*., 1992; Gaur *et al*., 1997). Drinking water pathway somehow linked with sewage pipe also contribute to different level of water contamination reported by (Clark *et al*., 1982).

Different level of contamination has been reported in drinking water through several studies but problem remains previous. In our environment bacteria are present everywhere, including surface and groundwater. Bacterial presence doesn't mean the water isn't safe always to drink, only a little number of bacteria are harmful for human and animal. These bacteria are called pathogenic and they can produce disease in human and animal body.

Total coliform bacteria mean a different kind of bacterial group, those are generally found in the environment, such as soil, vegetable, water etc. This group of bacteria are usually not harmful. The subgroup of Total coliform bacteria is Fecal coliform bacteria. They exist in a large number in the feces & intestine of the living being. The presence of fecal coliform bacterial in drinking water strongly indicates as an evidence of animal waste or sewage contamination & there has a huge risk that harmful bacteria are present. These Microbes can cause diarrhea, cramps, nausea, headaches or other symptoms & also can cause long-term health effects of older people, young children & infant also.

The most important species of genus *Salmonella* is *S. typhimurium* & *S. typhi* for human health being (Lan *et al*., 2009). Typhoid fever is not so common in developed countries but it is considered as a demolishing disease which causes several health issues in various region of Asia, Africa and South America. In 2000, more than 21 million cases were estimated, with up to 2,00,000 death results & it became a global burden (Crump *et al*., 2004). Water borne diseases are commonly accompanied by *Salmonella* are increased due to faecal contamination of water bodies (Caterina *et al*., 2012). Another

genus *Shigella* consists of four species, which are *S. sonnei*, *S. flexneri*, *S. dysenteriae* & *S. boydii*. All of those are responsible for bacillary dysentery or shigellosis, a disease that causes high fever, hemorrhagic dysentery & neurological disturbances (Sansonetti *et al*., 2001).

Shigella spp. are normally found in water polluted with human excrement. When *Salmonella* found in drinking water, it indicates the presence of human faecal contamination. This bacterium has its major pathogenicity, for this reason it has a great significance in the public health sector (Karanis *et al*., 2002).

Identification of *E. coli* and *Salmonella* from contaminated drinking water is so important. This study is also based on those two bacterial identifications.

Objectives

The present study has the following objectives regarding the current situation of microbial contamination of water.

- Isolation and identification of *Salmonella spp.* and *E. coli* from potable water in and nearby area of SAU campus
- Determination of the prevalence of bacterial load in drinking water collected from the study area.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

water is a universal solvent, it dissolves organic and inorganic compounds which take part in balancing metabolic reactions, maintaining the molecular framework, working on thermoregulation, maintaining transport of nutrients, stabilize plasma membrane and also maintaining body volume/weight. It is a vital component of all cells and it is prerequisite for all the living life on earth. Quality assessment of a vital element like water is very important. To conduct the standard of a safe water, it is also important to detect the organism present on it. This study is mainly based on this.

2.1 Isolation and identification of bacteria

Maggy *et al.* (2006); they studied to detect the prevalence of enteric pathogens those can produce disease in the communities. They collected bacterial isolates from several surface and groundwater sources & the community used their daily needs of water. They isolated the bacteria by culture media and by the 20E API kit, and *E. coli*, *Salmonella* and *Vibrio cholerae* were presumptively obtained and then analyzed by PCR (Polymerase chain reaction). They observed enteropathogenic, enterotoxigenic and entero-invasive *E. coli*, toxigenic *V. cholerae* also observed from the water samples. By all of this observation mention that the pathogenic bacteria which are present can cause a serious health risk to consumers.

Breede (2006), this study showed that average Total coliform/100 ml in water is 93 whereas, expected level was less than 1.

Sirajul *et al.* (2007); this study described the extent of faecal pollution during and after the 2004 flood in Dhaka. A total of 300 water samples were collected from 20 different drinking water sources in Kamalapur, Dhaka City. The level of faecal contamination was estimated using measurements of faecal indicator bacteria (total coliforms, faecal coliforms and faecal streptococci) and isolation of *Vibrio cholerae* was also carried out. Total dissolved solids, dissolved oxygen, hardness, chloride and PH were also monitored. The unacceptable level of contamination of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) was recorded ranged from 23·8% to 95·2%, 28·6% to 95·2% and 33·3% to 90·0% respectively.

Jessica *et al*. (2010); the 96 shallow & 55 deep tube wells water was observed from two villages of Bangladesh during the wet and dry season. They observed to detect the arsenic and *E. coli* from those sample, The fecal indicator *E. coli* was detected in 43% of shallow wells & 12% in deeper wells. The shallow wells contained 61% of *E. coli* during the wet season, which is more than the dry season that is 9%.

Thongchankaew *et al*. (2011); in this study, the predominance and diversity of *Vibrio spp.* was investigated over a 12-month period in a coastal lagoon, Songkhla Lake, in southern Thailand. Water samples were collected at 2 stations in the estuary near Yor Island in Songkhla Lake. The predominant *vibrios* were detected by a culture-based method. The highest numbers of total vibrios and *Vibrio parahaemolyticus* in both areas were observed during the summer. Variations in Vibrio species were observed with changes in salinity. *Vibrio parahaemolyticus* and *V. cholerae* non-O1/non-O139 were detected during the rainy season when the salinity dropped to nearly 0 parts per thousand. In both areas, *V. alginolyticus* was the most prominent species detected by the culture method.

Subhashree *et al*. (2012); they isolated bacteria from 18 different water samples collected from 3 different stations of Chilika lagoon in the State of Odisha of India. They took one ml of each water sample which was subjected through serial dilution to obtain 10^{-8} dilution. After that 1ml of 10^{-8} diluted sample was mixed with 10 ml of sterilized Nutrient broth medium and then it was incubated at 37 °C in incubator for 24 h. Then they picked up a loop-full of microbial culture from different colonies & then they streaked separately on the different agar-gelled sterilized media and the plates were incubated at 37 °C for 24 h for isolation of pure culture. They recorded colony characteristics such as color, appearance, and shape of the isolates. Other biochemical tests had also been performed by this paper. Then they indicate that the isolates are closely related to *Shigella dysenteriae*, *Streptococcus lactis*, *Bacillus cereus*, *Klebsiella pneumoniae*.

Peter *et al*. (2012); Bangladesh is underlain by shallow aquifers in which millions of drinking water wells are emplaced without annular seals. Fecal contamination has been widely detected in private tubewells. To evaluate the impact of well construction on microbial water quality 35 private tube wells (11 with intact cement platforms, 19 without) and 17 monitoring wells (11 with the annulus sealed with cement, six

unsealed) were monitored for culturable *E. coli* over 18 months. Additionally, two 'snapshot' sampling events were performed on a subset of wells during late-dry and early-wet seasons, wherein the fecal indicator bacteria *E. coli*, Bacteroidales and the pathogenicity genes eltA (enterotoxigenic *E. coli*; ETEC), ipaH (Shigella) and 40/41 hexon (adenovirus) were detected using quantitative polymerase chain reaction (qPCR). No difference in *E. coli* detection frequency was found between tube wells with and without platforms. Unsealed private wells, however, contained culturable *E. coli* more frequently and higher concentrations of fecal coliform bacteria than sealed monitoring wells ($p < 0.05$), suggestive of rapid downward flow along unsealed annuli. As a group the pathogens ETEC, *Shigella* and *adenovirus* were detected more frequently (10/22) during the wet season than the dry season (2/20).

Samie *et al*. (2012); they studied to detect the bacterial organism isolated from household drinking water. They collected samples from the household in the area of Makhado Municipality of the Limpopo province of South Africa. They collected sample from the storage containers in their homes in sterile 500-ml bottles and transported to the laboratory within two hours after collection. Collected samples were transported on ice to the Microbiology Laboratory. The membrane filtration method was used for all counts. Plate count agar, m-Endo agar, and m-FC agar were used for heterotrophic counts, total coliform counts (TCs), and faecal coliform counts (FCs), they also performed *Salmonella-Shigella* agar and m-Endo agar to isolate *Vibrio*, *Campylobacter*, *Salmonella/Shigella*, and *E. coli* respectively. Subculturing was also performed to obtain pure colonies by streaking into fresh plates. Different biochemical tests also performed by them. The results for HPC ranged from 42 colony-forming unit cfu/100 ml to 8.4×10^8 cfu/100 ml. From the 270 water samples collected, 134 organisms were isolated comprising 37 different bacterial species. The most common isolates included: *Pseudomonas spp.* (16.3%), *Shigella spp.* (14.2%), *Acinetobacter lwoffii* (7.5%), *Enterobacter cloacae* (7.5%), *Yersinia enterocolitica* (6.7%), and *E. coli* (6.0%).

Acharjee *et al*. (2013); study was carried out to assess the quality of municipal water supplied by DWASA at MODS zone in Dhaka City. Which was consisting of 78 water pump station. Water samples were randomly collected from 40 different locations between April, 2011 and March, 2012. Among them, ten were from selected pump

stations (supply points) and another 30 were from the household points (consumer points).

In this study, a aliquot of 100 ml of each sample was filtered through a 0.45 membrane filter and placed on the nutrient agar and membrane fecal coliform agar plates for the estimation o100 ml of each sample was filtered through a 0.45-mmmembrane filter and placed on the nutrient agar and membrane fecal coliform agar plates for the estimation of HPC and FCC, respectively. For HPC, plates were incubated at 37°C for24 h while for estimating the fecal coliforms, incubation at 44.5°Cfor 24 h was carried out.

High numbers of pathogenic bacteria were observed both in supply points and consumer points. Fecal coliforms (0.001–0.066 cfu/ml), *Klebsiella* spp. (0.012–0.026 cfu/ml) and *E. coli* (0.08–0.098 cfu/ml) were found only in samples of consumer points and completely absent from the supply points. *Salmonella* and *Shigella* spp. were found to be present in 15 and 24 samples, respectively, in the consumer points; whereas in the supply points, their predominance was very low. Most of the samples, both from supply points and consumer points, were contaminated with *Aeromonas* spp.; *Pseudomonas* spp. was found in 14 samples whereas *Staphylococcus* spp. was found in 22 samples in which three were from supply points.

Ahmed *et al*. (2013); they collected Forty-six bottled water samples representing 16 brands from Dhaka, Bangladesh was tested for the numbers of total coliforms, fecal indicator bacteria (i.e., thermotolerant *E. coli* and *Enterococcus spp*.) and potential bacterial pathogens (i.e., *Aeromonas hydrophil*, *Pseudomonas aeruginos*, *Salmonella*, and *Shigella spp*.). Among the 16 brands tested, 14 (86%), ten (63%) and seven (44%) were positive for total coliforms, *E. coil* and *Enterococcus spp.*, respectively. Additionally, a further nine (56%), eight (50%), six (37%), and four (25%) brands were PCR positive for *Salmonella* and *Shigella spp.* respectively. Their study results suggested that microbiological quality of bottled waters sold in Dhaka, Bangladesh is highly variable. To protect public health, stringent quality control was recommended by this study for the bottled water industry in Bangladesh.

Faisal *et al*. (2013); traditional reservoirs for water storage are important systems of water supply in rural areas of Morocco. This study aimed to assess the physicochemical and bacteriological quality of stored water. They used several culture media like-

Lactose TTC agar with Tergitol, for coliform counts, Bile aesculin agar for Group D streptococci counts, Baird Parker agar (BP-Agar) was used to select *S. aureus*. A critical contamination level was detected 8 × 105 cfu/100 ml in water.

Urmi *et al*. (2015); they attempted to detect the possible presence of *Vibrio parahaemolyticus* in the freshwater sediments with an objective to demonstrate its pathogenic divergence as well as the dissemination potential within the aquatic environment. It is a well-known marine bacterium with a pandemic effect of causing enteric diseases. Isolates of *V. parahaemolyticus* were screened from freshwater sediments by the river sides around Dhaka City, and their genotypic traits were chalked out through *toxR*, *tdh*, and *trh* gene-specific amplification. Pulsed field gel electrophoresis analysis detected 65% similarity between the reference strain *V. parahaemolyticus* O3:K6 and the O1:K56 isolates. Since O1:K56 is known to be a serovariant of O3:K6 which imparts the pandemic potential, the unlikely presence of O1:K56 strains in the freshwater samples in our study further demonstrates the possible eco-pathological impact.

Chandra *et al*. (2016); Jaipur city is one of the largest and fast developing cities of state Rajasthan, India. Majority of population in the city depends on piped water supplies for drinking and other domestic purposes. This study was to assess the microbiological and physicochemical quality of drinking water supplied in Jaipur. Water samples from twelve areas were collected and evaluated for physicochemical contaminants such as pH, electrical conductivity, fluoride, iron, nitrate, residual chlorine, total dissolved solids, total hardness and turbidity. The samples were also evaluated for eight most commonly found bacterial pathogens. Pathogenic bacteria were detected using polymerase chain reaction-based assays. Majority of parameters except few were found within bureau of Indian standards safety limits. By the observation, *Pseudomonas* was the most prominent bacteria and was detected in all locations. *E. coli*, *Aeromonas* and *Shigella* were detected in three of the twelve locations, whereas *Campylobacter* was detected in two of the locations. *Vibrio*, *Salmonella* and *Arcobacter* were not detected in any of the locations.

Mohammed, A, N. (2016); they collected 120 samples from different sources like, 65 from tap, 25 from underground and 30 from surface water source, which was used as a drinking water for cattle. For isolation and identification of pathogenic bacteria,

collected samples were cultured and also using serological techniques and PCR. By this observation in surface water *E. coli* was isolated as the highest prevalent bacteria (56.7%) then *Staphylococcus aureus* (36.7%), *Salmonella* (26.7%), *Streptococcus faecalis* (23.3%), *Shigella flexneri* (16.7%), *Proteus spp.* (16.7%), and *Klebsiella pneumonae* (10.0 %). The higher contamination was observed from the surface drinking water than the tap water.

Sujan *et al*. (2017); this study detected the water quality of the Karnaphuli river, located in the southern region of Bangladesh. A large population living in the area depend on this river for drinking, household, or recreational water. By this study Several standard physicochemical and microbiological parameters were used to determine the level of pollution. The mean values of the physicochemical parameters were significantly different from those of the WHO guideline values. The microbiological examination of the river water as determined by measuring total coliform and total fecal coliform demonstrated that the water is highly contaminated by pathogenic bacteria. Coliform and fecal coliform counts were also significantly higher than those of the standards recommended by WHO. The results indicate that the river is highly polluted by various chemical pollutants and pathogenic bacteria. Regular monitoring and immediate measures are required to reduce risks for public health and safety.

Osvalda *et al*. (2017); Apulia is a region in southeastern Italy, agriculture industry is widespread in this area. For this agricultural activity, a large amount of water is needed for irrigation purpose in this area. There has no significant lakes or rivers for irrigation. So, groundwater covers 75% of irrigation demand for the local population & this study observed the bacteriological quality of groundwater used for irrigation. After collected 182 groundwater sample for observation & they had done several cultural and biochemical tests. By the observation 141 samples were contaminated with the presence of fecal bacteria like *E. coli*, *Salmonella*, *Enterococci* & total coliforms. They also mentioned the bacteria included in Ministerial Decree in 185 samples, 35(19.2%) samples water wasn't suitable for irrigation. the water from 35 (19.2%) wells was unsuitable for irrigation purposes & another 147 sample water sources were considered as suitable.

Sohana *et al*. (2018); in this study they collected 38 samples of supplied jar water to determine the microbial contamination and physiochemical properties. They did TVC,

MPN and different biochemical test for identification & enumeration. The range of total bacterial count was (1.5×10^2 –1.6×10^4) cfu/ml. The total coliform count (TCCm) was recorded (14–40) in 100 ml of water samples. The presence of total coliform and fecal coliform was 26.32 and 18.42%, respectively, in PCR analysis but in biochemical test those were 18.42 and 15.78%, respectively. A total of 11 bacterial species: *Enterobacter aerogenes*, *E. coli*, *Aeromonas*, *Bacillus sp.*, *Cardiobacterium*, *Corynebacterium*, *Clostridium*, *Klebsiella sp.*, *Lactobacillus*, *Micrococcus sp.*, *Pseudomonas sp.* were found. This study indicates that some of the drinking jar water samples were of poor quality which may increase the risk of water-borne disease.

Harada *et al*. (2018); they investigated the pathotypes of *E. coli* in sanitary wastewater and stored drinking water in a slum of Bangladesh and 621 *E. coli* isolated for identification of pathotypes. For this they performed PCR and dual index sequencing. By their observation the pathogenic *E. coli* were significantly in waste water (18.6%) and drinking water (1.7%). Their findings indicated that the sanitary waste water was heavily contaminated with pathogenic *E. coli*, which can cause a great health risk & the drinking water could be contaminated not only by human sources but also others.

Ouf *et al*. (2018); water quality and bacterial contamination from 18 drinking water municipal plants in three locations at Giza governorate were investigated. The average total count of bacteria detected after four stages of treatments in the investigated plants was 32 cfu/1 ml compared to 2330 cfu/ml for raw water, with a reduction percentage of 98.6. Although there is a relatively high removal percent of bacterial contamination from the water sources, however, several bacterial pathogens were identified in the produced water prepared for drinking including *Enterococcus faecalis*, *E. coli*, *Pseudomonas aeruginosa*, and *Shigella spp.* After 3 days of water incubation at 30 °C, the number of bacterial endotoxins ranged from 77 to 137 ng/ml in the water produced from the municipal plants compared to 621–1260 ng/ml for untreated water. The main diseases reported from patients attending different clinics and hospitals during summer 2014 at the surveyed locations and assuredly due to drinking water from these plants indicated that diarrheas and gastroenteritis due to *E. coli* and *Campylobacter jejuni* constituted 65.7% of the total patients followed by bacillary dysentery or shigellosis due to *Shigella spp*. (7.9%) and cholera due to *Vibrio cholera* (7.2%).

Yirenya *et al.* (2018); Cholera is a global public health problem with high endemicity in many developing countries in Africa. In 2014, Ghana experienced its largest epidemic with more than 20,000 cases and 200 deaths; most of it occurred in the Accra Metropolitan Area (AMA). Ghana's disease surveillance system is mainly clinically based and focused on case detection and management. Environmental exploration for the etiological agents is missing from the surveillance strategy. This study therefore assessed the occurrence of toxigenic *Vibrio cholerae* in water storage systems in selected high-risk areas in the AMA area prior to the 2014 outbreak. Three hundred twenty water samples from 80 households' water storage systems were analyzed for toxigenic Vibrio cholerae using the bacterial culture method. Presumptive *V. cholerae* was isolated from 83.8% of households' water storage systems. The viable cells ranged from 1 to 1400 cfu/100 ml.

Emad *et al*. (2019); drinking water quality plays a remarkable role in human infections and diseases. This study used polymerase chain reaction (PCR) techniques to detect bacterial pathogens. In addition, a physicochemical analysis was performed on drinking water samples from several sources. A total of 123 drinking water samples were collected from different areas in the Jazan region in Saudi Arabia: ground water (40 samples), bottled water (15 samples), tap water (52 samples), and water purification shops (16 samples). To isolate the bacterial pathogens, the water samples were spread on Nutrient and MacConkey agar media, and the grown pathogens were then identified by the 16S ribosomal RNA technique. In 87 (70.7%) of the 123 drinking water samples, there was no pathogen growth on the two-culture medium. However, 36 (29.3%) of the samples were found to be contaminated with bacteria. The contamination in the water may be occurring at the reservoirs rather than the water sources.

Al-quraan *et al.* (2020); they studied in the area of Jordan, which is one of the lowest countries in the world in terms of water resources. The reuse of treated wastewater is an important alternative to supply agricultural demands for water. In this study, bacterial contamination was evaluated in six sites of this region. The isolated pathogenic bacteria were *Salmonella sp., Shigella sp., Bacillus cereus* and *Staphylococcus aureus*. This study showed high contamination with many pathogenic bacteria and coliforms.

Aftab *et al.* (2020); this study attempted to isolate and quantify the microorganisms from various jar and bottle water samples collected from various areas of Dhaka City. Out of the eighteen samples studied, ten were jar water samples and eight were bottled water samples. They performed cultural and biochemical tests for observation. The range of total viable bacterial count (TVBC) in these samples ranged from 10^2 to 10^5 cfu/ml. Specific pathogens such as, *Salmonella*, *Shigella spp*., *Vibrio spp*. and fecal coliforms could not be found in these samples. However, coliforms could be detected in 10 samples.

2.2 Prevalence of *E. coli* and *Salmonella*

Garba *et al*. (2009); they collected 180 water samples, 60 were from well, 60 from tap water and another 60 from packaged for the identification of *E. coli* and for estimating total coliform counts. After biochemical analysis of those samples indicated 63 confirmed presence of *E. coli*, prevalence for well water 45.5%, tap water 23.3% & packaged water was13.3%.

Jokinen *et al*. (2010); 342 samples were collected from surface water, 91 sample indicated *Salmonella* presence and 8 samples presence *E. coli*

Ahmed *et al*. (2011); this study detects the prevalence of *Salmonella spp* is 13.34% from drinking water in Dhaka City.

Anera *et al*. (2014); they collected 140 water samples to determine them bacterial quality. Among these drinking water samples total coliforms bacteria, fecal coliforms bacteria and *E. coli* prevalence was 21.4%, 18.6% and 17.8%.

Moges *et al*. (2014); they collected 60 samples from processed hospital waste water, they examined and found several types of bacteria among them 11.5% was *E. coli.*

Shahidul *et al*. (2014); prevalence of *Salmonella* was detected 35% in case of restaurant water from Dhaka City.

Melissa *et al*. (2015); this study was performed to conduct the microbiological parameters of environmental samples of fresh water from rivers of Curitiba and its metropolitan area in Parana State, Brazil. They detected the prevalence of *E. coli* was 19.43%.

Khan *et al*. (2016); this study observed 100 samples & 17 samples was positive for total coliform bacteria.

Kumarasingam *et al*. (2016); they studied 140 waste water sample and recycle them for 2 hours, isolated several bacteria and the prevalence of *E. coli* was 16.42%.

Osvalda *et al*. (2017); by this observation 182 water sample was collected which was used for irrigation purpose, the prevalence of *E. coli* and *salmonella* was 77.5%.

Sarkar *et al*. (2019); they collected 55 fecal samples from deer and 6 water samples from 2 lakes, they isolated 32 *E. coli* among them 6 was from 6 number of samples of 2 lakes water.

Mahagamage *et al*. (2020); in this study 72 ground water sample were collected and 45 Surface water sample was examined. Almost all the samples were contaminated with *E. coli* and in case of ground water 17 samples were present *Salmonella* and 26 samples were present *salmonella* for surface water.

Aftab *et al*. (2020); they observed 18 samples of bottled and jar water from Dhaka City and *E. coli* was present in 8 number of samples and the prevalence was 44.44%.

Jamil *et al*. (2020); they observed 425 primary schools water samples from ten districts of Sindh, Pakistan. They had used Quantitative Microbial Risk Assessment technique to detect the possibility of infection among schoolchildren by the consumption of those water. They found around half of the water samples from those schools were contaminated with *E. coli* (49%) & *Salmonella* (53%).

CHAPTER 3

MATERIALS & METHODS

CHAPTER 3

MATERIALS & METHODS

This study was conducted to the Microbiology and Parasitology laboratory of the Faculty of Animal Science and Veterinary Medicine in Sher-e-Bangla Agricultural University, Dhaka-1207. The whole experiment was performed during the period of January 2021 to August 2021.

3.1 Different types of materials and chemicals

3.1.1 Glass wares and others instrument

- Collecting vial
- Test tube
- Test tube holder/ rack
- Conical flask
- Spirit lamp
- Cotton, Foal paper
- Petri dish
- Eppendorf tube
- Pipette
- Micropipette
- Measuring cylinder
- Electric balance machine
- Electric stirrer
- Glass Spreader
- Streaking loop
- Incubator
- Laminar air flow
- Refrigerator
- Autoclave

3.1.2 Chemical appliances

- PBS (Phosphate buffer solution)
- 70% Alcohol
- Distilled Water

- 3% Hydrogen per Oxide (H_2O_2)
- Normal saline solution
- 50% Buffered Glycerol Saline
- Other common laboratory chemicals

3.2 Sampling area

Table 1: Number of samples collected from different areas

Sl. No.	Name of Area	Number of Sample
01	Collage gate street food shop	3
02	Mini market	3
03	Shiraj-ud-doula Hall	3
04	Agri. Faculty Bhaban	3
05	Krishokrotno Sheikh Hasina Hall	3
06	Food Shop gate-2	3
07	Tea stall gate-2	3
08	Agargaon Bazar	3
09	Paka Market	3
10	Pitha store near gate-1	3
11	Food shop near gate-1	3
12	Tea stall near gate-1	3
13	Kabi kazi Nazrul Islam Hall	3
14	Begum Sheikh Fazilatunnesa Mujib Hall	3
15	Jhalmuri shop	3
16	Fuska shop	3
17	Sheikh Lutfur Rahman Hall	3
18	Food shop near gate-2	3
19	Sheikh Sayera Khatun Hall	3
20	Sheikh Kamal Bhaban	3
	Total	60

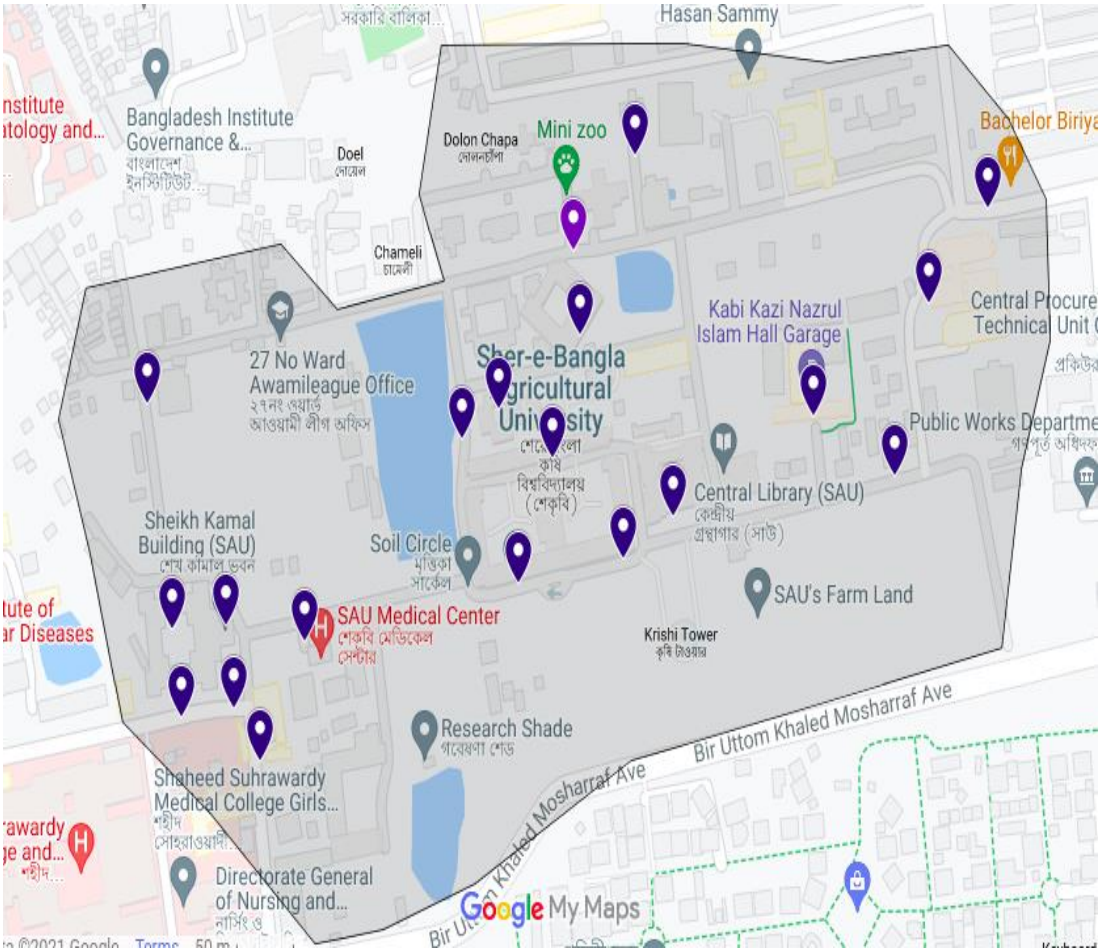

Figure 1: Geographical area of sample collection

3.3 Water sample collection

A total of 60 drinking water samples were collected from different areas of SAU campus and its surrounding area of Dhaka City in Bangladesh in sterile vial .1000 ml of each sample were collected and they were then transferred to the microbiology laboratory in SAU campus. Hygienic and aseptic practices were executed during sampling of drinking jar water.

3.4 Duration of the experiment

Water samples were collected from 20 sites from this study area and the study was performed at the laboratory of the Department of Microbiology & Parasitology, Sher-e-Bangla Agricultural University, Dhaka- 1207 & the time duration was from January 2021 to August 2021.

3.5 Experimental Design

Collection of samples from different areas in and around SAU campus

⬇

Physical examination of each sample was performed (color, odor, turbidity, PH etc.)

⬇

Then the sample first inoculated into Nutrient Broth for growth of bacteria & incubated at 37^0 C temperature for overnight

⬇

Performed 10-fold dilution of the inoculum by using PBS solution

⬇

Then spread into Nutrient agar and MacConkey agar media

⬇

Kept in incubator for 24 hrs at 37^0 C temperature

⬇

Then cultured in SS agar media & Eosin Methylene Blue agar media by streak plate method

⬇

Kept it for incubation 24 hours at 37^0 C temperature

⬇

Observation of the colony morphology

⬇

Then performing gram staining to identify staining properties (gm^{+ve} or $gm^{-ve)}$

⬇

Then performing microscopic examination to identify the shape of the bacteria

⬇

Then different bio-chemical test was performed & Nutrient Broth inoculum was stored for further use.

Figure 2: Schematic illustration of the experimental design

The experimental design of this study is to detect the bacterial load, isolation and identification of bacteria exist in drinking water sample based on cultural properties, morphological characteristics and biochemical analysis.

Samples were collected from the following areas and transferred in the laboratory by the following of aseptic measure. After collection they were taken into Nutrient Broth and stored in 4^0 C temperature for farther use. Then taken into PBS solution and performed serial dilution to spread in different culture media. Pure cultures of the organisms were subjected to morphological, cultural properties and biochemical analysis.

3.6 Sterilization & preparation of instruments and glass wares

Glass wares were cleaned by using 2% sodium hypochlorite solution & rinse in it. Soaked for overnight in a dish washing detergent and then washed it. Round brush was used for cleaning of test tube, collecting vial etc. & cleaned the other instruments by washing under running tap water. Kept it for air dry. After dried all the instrument wrapped with the foal paper and got ready for autoclaving. Autoclaved at 121^0 C temperature 15lbs. per square inch for 15 minutes. After autoclaving the instruments and glass wares were kept for air dry.

3.7 PBS (Phosphate Buffered Saline) Solution Preparation

PBS (Phosphate Buffered Saline) solution prepared by using-

- 0.2 gm. of potassium chloride
- 2.89 gm. of disodium phosphate
- 8 gm. of sodium chloride and
- 0.2 gm. of potassium hydrogen phosphate

Those were mixed in 1000 ml of distilled water. With an electric magnetic stirrer, the solution was mixed and heated to dissolve properly. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. Then stored it for further use.

3.8 70% Alcohol Preparation

For preparation of 70% alcohol, pure ethanol (100% ethanol) was used. Through a measuring cylinder 700 ml pure ethanol was mixed with 300 ml of distilled water.

3.9 50% Buffered Glycerol Saline Preparation

For preparation of 50% Buffered Glycerol Saline, 8.3 gm of Base of buffered glycerol saline was added in 700 ml distilled water. Then added 300 ml glycerol in it. Then heated it to dissolve completely. After mixing well, it was dispensed in tubes, which was screw capped or in containers. Autoclaved for sterilization at 121^0 C temperature at 15 lbs./ $inch^2$ pressure for 15 minutes.

3.10 Media used for bacterial observation

3.10.1 Solid Media

3.10.1.1 Nutrient Agar Media

For preparation of Nutrient agar media, in a conical flask 2.8gm powder of Nutrient agar dissolved in 100 ml distilled water and boiled to dissolve completely through electric stirrer. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving media was poured into petridishes for solidification and the quantity of media for medium size petridish was 10ml/ petridish and for large size the amount was 15ml/ petridish. Then inoculated and incubate at 37^0 C temperature for overnight.

3.10.1.2 MacConkey Agar Media

Due to preparation of MacConkey agar media, in a conical flask 51.53 gm powder of MacConkey agar dissolved in 1000 ml distilled water and boiled to dissolve completely. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving media was poured into petridishes for solidification and the quantity of media for medium size petridish was 10ml/ petridish and for large size the amount was 15ml/ petridish. Then inoculated and incubate at 37^0 C temperature for overnight.

3.10.1.3 *Salmonella-Shigella* (SS) Agar Media

Due to preparation of SS agar media, in a conical flask 63.02 gm powder of SS agar dissolved in 1000 ml distilled water and heated to dissolve completely. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving media was poured into petridishes for solidification and the quantity of media for medium size petridish was 10ml/ petridish and for large size the amount was 15ml/ petridish. Then inoculated and incubate at 37^0 C temperature for overnight.

3.10.1.4 Eosin Methylene Blue (EMB) Agar Media

For preparation of EMB agar media, in a conical flask 35.96 gm EMB agar powder dissolved in 1000 ml distilled water and boiled to dissolve completely. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving media was poured into petridishes for solidification and the quantity of media for medium size petridish was 10ml/ petridish and for large size the amount was 15ml/ petridish. Then inoculated and incubate at 37^0 C temperature for overnight.

3.10.2 Liquid Media

3.10.2.1 Nutrient Broth

Nutrient broth is a liquid media used as a primary media for inoculation and initial growth of bacteria occurred here. For preparation of Nutrient broth media, in a conical flask 13.0gm Nutrient broth powder dissolved in 1000 ml distilled water and heated to boil and to dissolve completely. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving media was allowed to be cool. After cooling it poured into the previously sterilized test tube and the quantity is 5ml/test tube. 100 micro litter sample was added into each test tube and incubated at 37^0c temperature for 24 hours.

3.10.2.2 Methyl-Red Voges-Proskauer (MR-VP) broth

For preparation of MR-VP broth media, in a conical flask 17gm broth powder dissolved in 1000 ml distilled water and heated to boil and to dissolve completely. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving, allowed to be cool. After cooling it poured into the previously sterilized test tube and the quantity was 2ml/test tube. Then it was allowed to incubate at 37^0 C for 72 hours.

3.11 Procedure for isolation of bacterial colony

3.11.1 Collection & transportation

Water samples were collected from following area by using collecting vial or conical flask & transported aseptically to the microbiology laboratory in SAU campus. Samples were used for observation within 24 hours after collection.

3.11.2 Physical examination

Physical examination was done by necked eye to detect the color, odor, turbidity, PH level (detect with paper stripes), specific gravity & presence of any foreign particles in each sample. If any difficulties found, they were discarded for further analysis.

Table 2: Physical parameter observation after sample collection

Parameters	Result
Color	Nil
Odor	Nil
Turbidity	Nil
PH	Near about 7
Any foreign particles	Nil

When all the physical parameters were same the further study were continued otherwise the sample were discarded.

3.11.3 Preparation of primary inoculum

100 micro littre of each sample was added into 5ml Nutrient broth containing test tube. This was then incubated at 37^0 C temperature for overnight.

3.11.4 Preparation of ten-fold serial dilution and spread plate method

For preparation of serial dilution, a series of Eppendorf tube was taken to the tube holder. 9 ml PBS solution was taken into each tube. 1 ml sample was then added to the first tube containing 9 ml PBS. Mixed it properly. Then 1 ml dilution discarded from the first tube and poured into second one. Again, mixed it properly. By this following method this was continued to the last one. For the last one 1 ml dilution discarded.

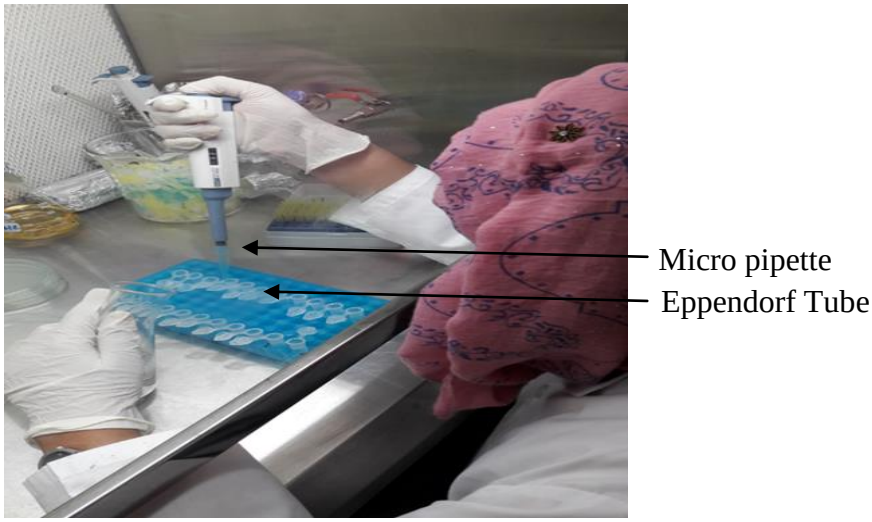

Figure 3: Performing Ten- fold dilution

Each dilution of each sample was then spread over the Nutrient Agar & MacConkey Agar media by spread plate method. A glass spreader with smooth edge was used for this spread plate method. 0.1 ml mixture was transferred into each petridish containing EMB & MacConkey Agar media. Glass spreader was sterilized by 70% alcohol and burned it in bunsen burner before using it for spreading in each media containing petridish.

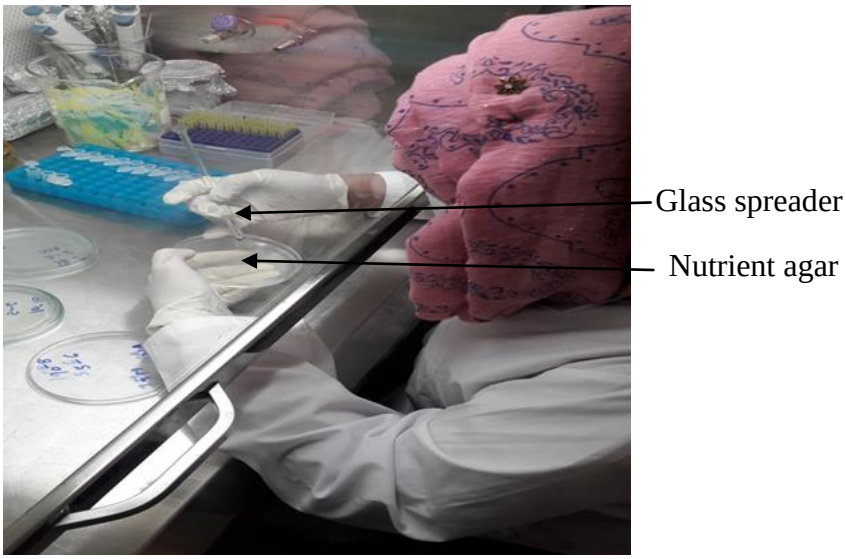

Figure 4: Spreading in agar media plate

After spreading, the petridishes incubated at 37^0 C temperature for overnight. Then each plate observed & 30-300 colonies containing plates were counted, More or less was discarded.

Figure 5: Colony counting

3.11.5 Isolation & streak plate method

The colony was then isolated and inoculate by streaking into different media to observe the growth of the bacteria in different media containing plate. Then incubated at 37^0c temperature for 24 hours.

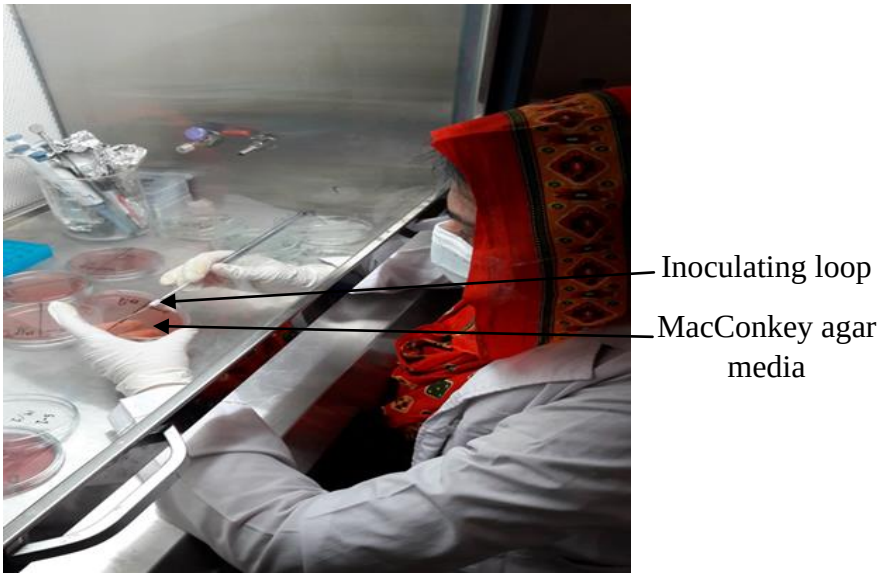

Figure 6: Streaking in MA agar media plate

3.11.6 Gram's staining procedure & Microscopic examination

For performing gram's staining procedure, a small colony was picked up with a sterile loop in a glass slide and smeared. Then heating gently to fixed on it. Applied crystal violet as a primary stain into the smear on glass slide & allowed to stain for two minutes. Then washed out with running tap water. Followed by few drops gram's iodine as a mordant was added and then washed out with running tap water. After washing rapidly added alcohol for decolorization. After this added safranin as a counterstaining and kept it for two minutes. Then again washed out with running tap water. Followed by blotted and air dried to examined under microscope (100X) by using immersion oil.

Figure 7: Performing Staining

Figure 8: Washing in running tap water

Figure 9: Microscopic observation

3.12 Bio-chemical analysis

3.12.1 Catalase Test

For catalase test culture was picked up with a sterile loop in a sterile glass slide. The test was performed by using 3% H_2O_2. Culture was taken to the agar plate with a sterile loop & a drop of 3% H_2O_2 was mixed on a clean sterile glass slide. Bubble was formed within a few seconds after adding H_2O_2 & it became positive. In case of negative there had no change after adding H_2O_2 with the colony.

3.12.2 MR Test

After preparation of medium 5ml/ test tube was taken and added the inoculum into this test tube. Incubated for 72 hours at 37^0c temperature. After incubation one drop of methyl red solution was then added in this test tube. After mixing, red color was indicated positive result & yellow color or no color change indicated negative result.

3.12.3 VP Test

2 ml of sterile glucose peptone water was inoculated with the 2 ml of test organisms. It was incubated at 37^0C for 48 hours. A very small amount of creatine was added and mixed. 3 ml of sodium hydroxide was added and shacked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In case of negative reaction there was no development of pink color.

3.12.4 Indole test

The organisms kept in a test tube which have 3 ml of peptone water containing tryptophan and cultured at 37ºC for 48 hours in incubator. After incubation 1 ml of diethyl ether was then added, Shaked and allowed to stand until the ether rises to the top. Then gently added 0.5 ml of Kovac's reagent by running down the side of the test tube. So that it formed a ring in between the medium and the ether layer. Observed for the development of ring. Development of a brilliant red colored ring indicated indole production. In negative case, no development of ring (Cheesbrough, 2006)

Figure 10: Different biochemical analysis

3.13 Preparation of Stock Culture

Stock culture was prepared for further analysis. stock culture was mixed with a medium prepared by 1ml of 50% sterilized glycerol in 1ml of pure culture in nutrient broth & was stored -20^{0}C for further use. It can be used for several years.

CHAPTER 4

RESULT & DISCUSSION

CHAPTER 4

RESULT & DISCUSSION

The result of this study has illustrated the quality of drinking water supplied in the selected regions of Dhaka City, where the site was Collage gate food shop, Minimarket, Shiraj-ud-doula hall, Agri. faculty bhaban, Krishokrotno Sheikh Hasina hall, Food shop inside campus gate, Food shop near gate-2, Agargaon bazar, Paka market, Pitha store near gate-1, Food shop near gate-1, Tea stall near gate-1, Kabi kazi Nazrul Islam hall, Begum Sheikh Fazilatunnesa Mujib hall, Jhalmuri shop, Fuska shop in campus, Sheikh Lutfur Rahman hall, Different food shop near gate-2, Sheikh Sayera Khatun hall, Sheikh kamal bhaban. Total quality of water was studied considering microbiological parameters and physico-chemical parameters. Coliform bacteria, *E. coli*, *Salmonella* are concerns of microbiological parameters.

4.1 Bacteriological examination

All the samples were firstly incubated in nutrient broth then, cultured in MacConkey agar media & Nutrient Agar Media to count the TCC of Coliform bacteria. The stock sample from Nutrient broth cultured in EMB agar media for isolation of *E. coli* and before counting the *E. coli* it was confirmed by biochemical test. The colony also cultured in *Salmonella- Shigella* Agar media and Eosin Methylene Blue Agar media.

4.2 Culture in Nutrient broth

All the sample cultured in Nutrient broth showed turbidity after incubated overnight which confirms the growth of bacteria.

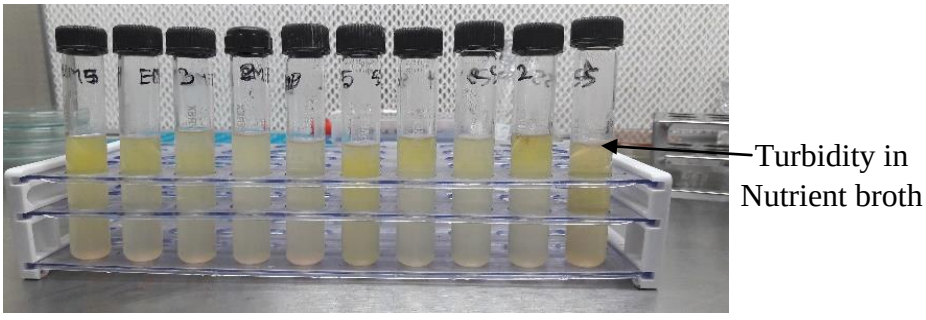

Figure 11: Turbidity grow in Nutrient Broth media

4.3 Culture in Nutrient Agar & MacConkey Agar media

After 10-fold dilution it was cultured in NA & MA Agar media & detected the Total viable count and Total coliform count.

Table 3: Total Viable Count (TVC) and Total Coliform Count (TCC) of the bacteria isolated from the water sample.

Name of Sample	Total Viable Count (cfu/ml)	Total Coliform Count (cfu/ml)
S_1	1.94×10^5	Nil
S_2	1.33×10^6	1.7×10^5
S_3	1.31×10^6	1.5×10^5
S_4	1.22×10^6	Nil
S_5	2.82×10^5	0.98×10^5
S_6	1.56×10^7	Nil
S_7	1.73×10^8	Nil
S_8	0.94×10^7	Nil
S_9	1.09×10^6	Nil
S_{10}	1.35×10^5	Nil
S_{11}	1.85×10^6	0.56×10^5
S_{12}	1.4×10^7	Nil
S_{13}	1.18×10^5	Nil
S_{14}	1.19×10^6	Nil
S_{15}	1.97×10^5	1.34×10^6
S_{16}	0.98×10^4	Nil
S_{17}	1.77×10^7	0.63×10^6
S_{18}	1.86×10^7	Nil
S_{19}	1.5×10^7	1.22×10^5
S_{20}	1.25×10^5	0.76×10^4
S_{21}	0.95×10^5	Nil
S_{22}	2.1×10^5	1.24×10^5
S_{23}	1.95×10^5	0.94×10^6

Name of Sample	Total Viable Count (cfu/ml)	Total Coliform Count (cfu/ml)
S_{24}	1.17×10^5	Nil
S_{25}	1.03×10^6	Nil
S_{26}	1.58×10^5	1.06×10^6
S_{27}	1.76×10^5	Nil
S_{28}	1.58×10^6	Nil
S_{29}	2.03×10^5	1×10^5
S_{30}	0.83×10^7	Nil
S_{31}	1.95×10^5	0.64×10^5
S_{32}	1.35×10^6	Nil
S_{33}	1.77×10^4	Nil
S_{34}	1.53×10^5	Nil
S_{35}	1.22×10^5	0.94×10^6
S_{36}	$1..07 \times 10^5$	Nil
S_{37}	0.98×10^6	Nil
S_{38}	$2.35x\ 10^4$	Nil
S_{39}	1.73×10^5	0.65×10^5
S_{40}	1.56×10^5	Nil
S_{41}	1.09×10^6	0.56×10^5
S_{42}	1.6×10^5	Nil
S_{43}	2.76×10^5	Nil
S_{44}	1.96×10^8	1.07×10^3
S_{45}	$o.79 \times 10^5$	Nil
S_{46}	1.57×10^4	0.55×10^5
S_{47}	1.89×10^5	Nil
S_{48}	1.91×10^5	0.74×10^5
S_{49}	1.17×10^6	Nil
S_{50}	2.39×10^6	Nil
S_{51}	1.11×10^5	Nil
S_{52}	1.8×10^6	Nil
S_{53}	0.7×10^5	Nil

Name of Sample	Total Viable Count (cfu/ml)	Total Coliform Count (cfu/ml)
S_{54}	2.23×10^5	0.93×10^4
S_{55}	2.46×10^6	Nil
S_{56}	1.94×10^4	Nil
S_{57}	1.76×10^5	Nil
S_{58}	2.04×10^5	Nil
S_{59}	0.96×10^6	Nil
S_{60}	1.67×10^5	Nil

In this study area of SAU campus and its nearby areas the Total viable count in Nutrient agar ranges from 0.7×10^5 to 2.82×10^5 and the Total coliform count in MacConkey Agar is ranges from 0.55×10^5 to 1.7×10^5 using 10-fold dilution method.

4.3.1 Colony formed in Nutrient agar

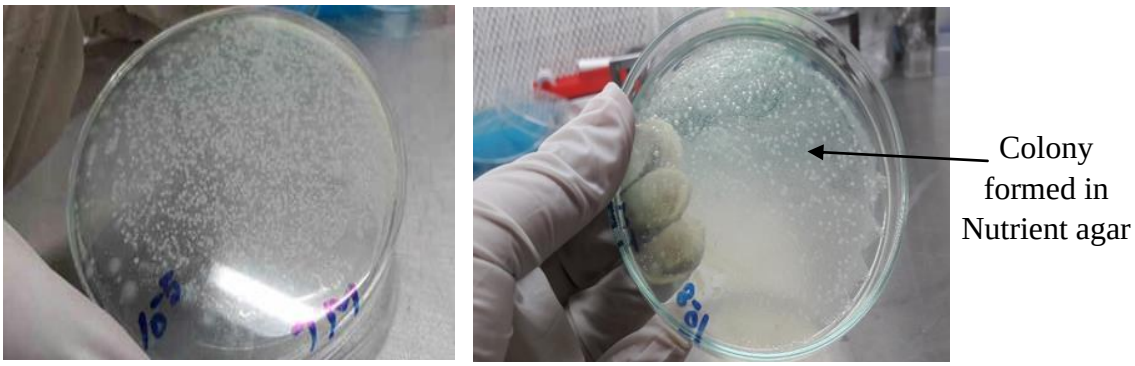

Figure 12: Colony count from Nutrient agar for estimation of TVC (Ten-fold dilution)

Total viable bacteria count was performed by enumerating of the inoculated 0.1 ml sample from each dilution into Nutrient agar (NA) using the spread plate method.

4.3.2 Culture in MacConkey agar media

In MacConkey agar media reddish to pinkish, whitish, dark centered brown colored colony was found which are characterized for coliform bacteria. Reddish, pinkish colony indicated the lactose fermenting coliform where whitish and brown color colony indicated the non-lactose fermenting coliform bacteria.

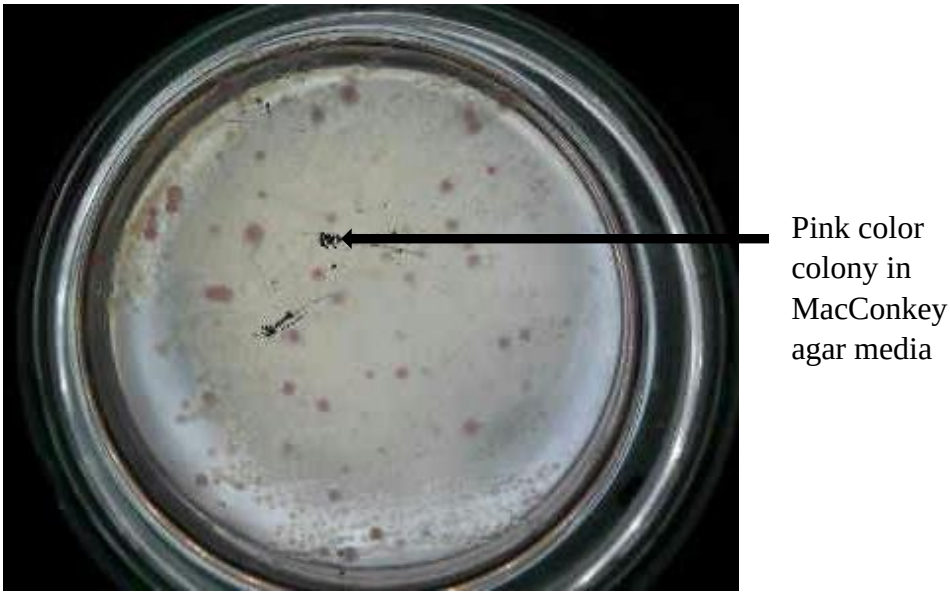

Figure13: Total coliform count by10 fold dilution method

Bacterial colony Plate count was performed between 30-300 colonies. Plate with more than 300 number of colonies were considered as too numerous numbers to count. On the other hand, plates with less than 30 number of colonies were considered as too few numbers to count.

4.4 Identification of *E. coli* in media

4.4.1 In MacConkey agar media: Produce bright pink color colony

Figure 14: Pink color colony in MacConkey Agar Media

4.4.2 In EMB agar media: Produce Greenish colony with metallic sheen

Cultured in EMB agar by streak plate method to identify *E. coli.* After Incubation for 24 hours at 37^0 c temperature, observed greenish red colony with faint/ rusty metallic sheen.

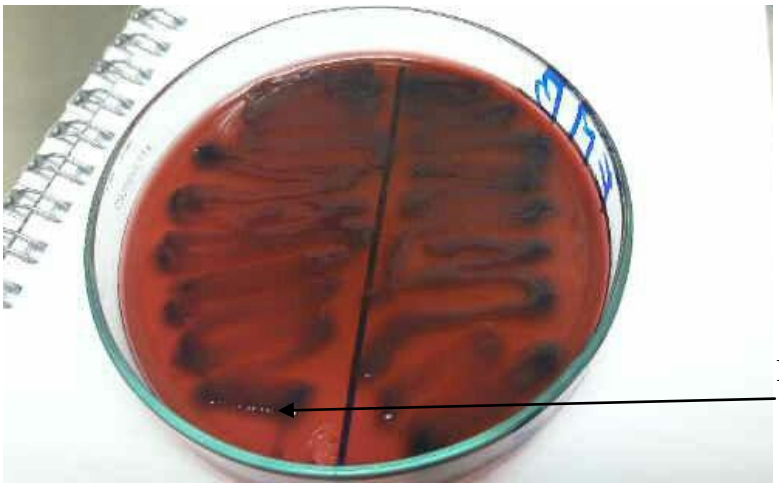

Figure 15: Greenish red color colony with faint metallic sheen(EMB agar)

4.5 Gram's staining and microscopic observation of *E. coli*

After gram's staining observed under light microscope. In case of *E. coli* observation it indicated gram negative, rod shaped,pink color & organism arranged as single or paired.

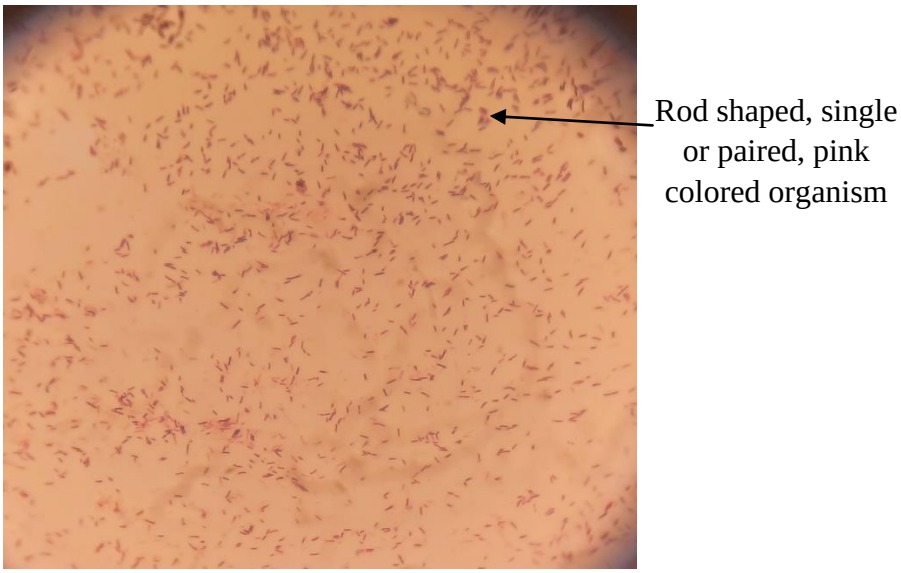

Figure 16: *E. coli* under microscope(100X)

4.6 Biochemical test to detect *E. coli*

4.6.1 Catalase test

In catalase test, bubble formation within a few seconds after added 3% H2O2 solution indicated the positive test for *E. coli*.

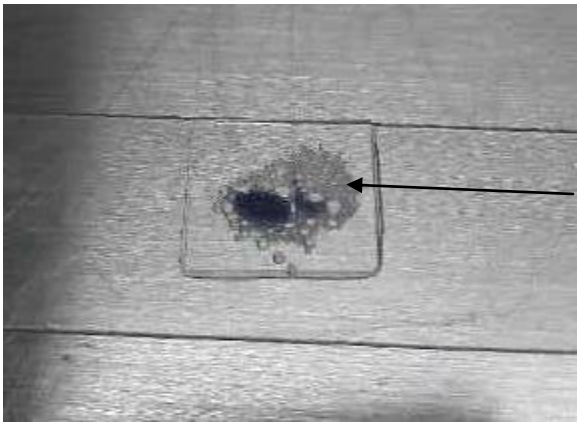

Figure 17: Bubble formation in catalase test

4.6.2 MR test

After preparation of media, added inoculum into test tube.Then Incubated for 72 hours. After incubation one drop of methyl red solution was added in this test tube. After that red color was indicated positive result & yellow color or no color change indicated negative result.

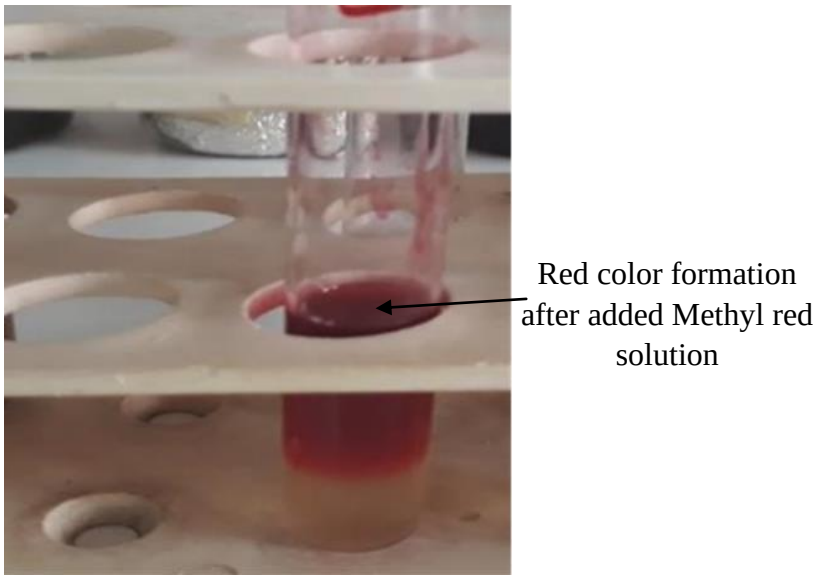

Figure 18: MR test (positive)

4.6.3 Indole test: After added Kovac's reagent, it forms a ring in between the medium and the ether layer. Development of a brilliant red colored ring indicated indole production. In negative case there is no development of red color (Cheesbrough, 2006)

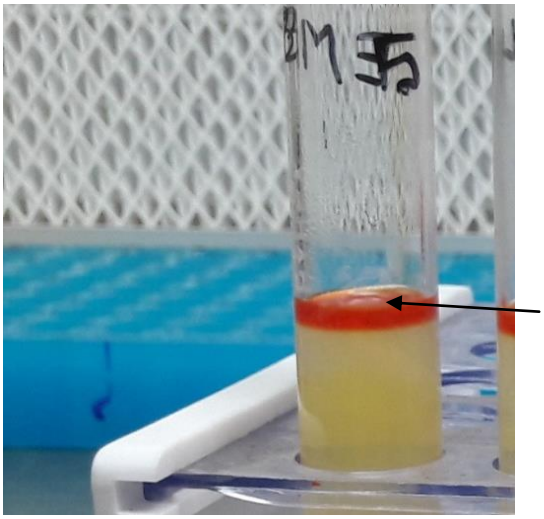

Figure 19: Indole test (positive)

The *E. coli* isolated and performed gram's staining and observed in Microscope to identify morphologically. Observed in different Media to identify colony characteristics and finally observed bio- chemically to identify bio-chemical characteristics.

Table 4: Growth properties, staining properties, microscopic observation and biochemical test of the *E. coli*

Properties	Observation
Growth Properties	Produce red to bright pink color colony in MA agar. Produce greenish red color colony with faint metallic sheen in EMB agar
Staining Properties	Gram negative
Microscopic Observation	Rod shape, pink color, arranged single or paired.
Biochemical Test	Bubble formation gives positive indication in Catalase test. Produce red color indicated positive result in MR test. No color change gives negative indication in VP test. Produce bright red to pink color ring gives positive indication in Indole test.

4.7 Identification of *Salmonella*

4.7.1 In EMB agar: Produce gray color colony

Figure 20: Gray Colony in EMB Agar

4.7.2 In SS Agar: Produce Black color colony

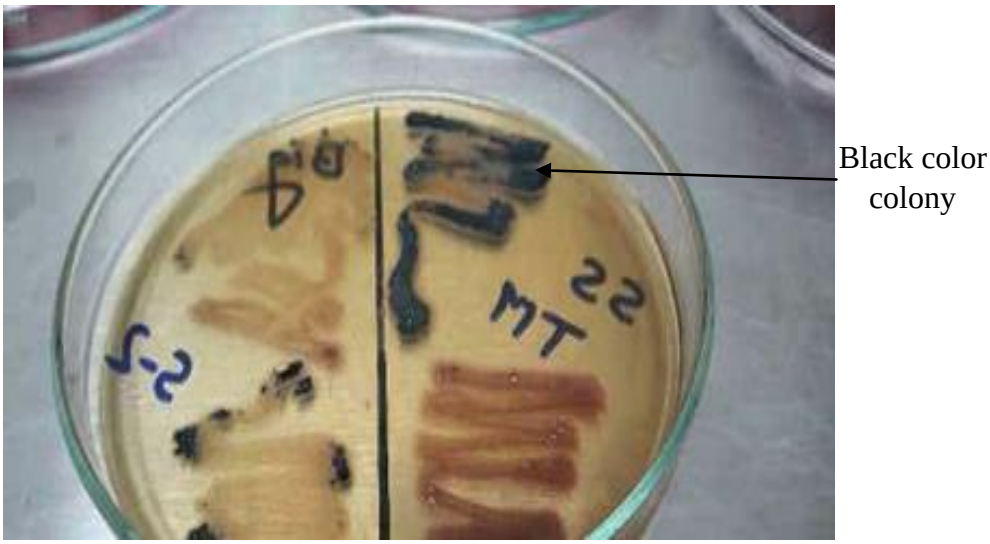

Figure 21: Black colony in SS Agar

4.8 Gram's staining and microscopic observation for *Salmonella*

After gram's staining observed under light microscope. In case of *Salmonella* observation, it indicated gram negative, rod shaped, pink color & organism arranged as single or paired.

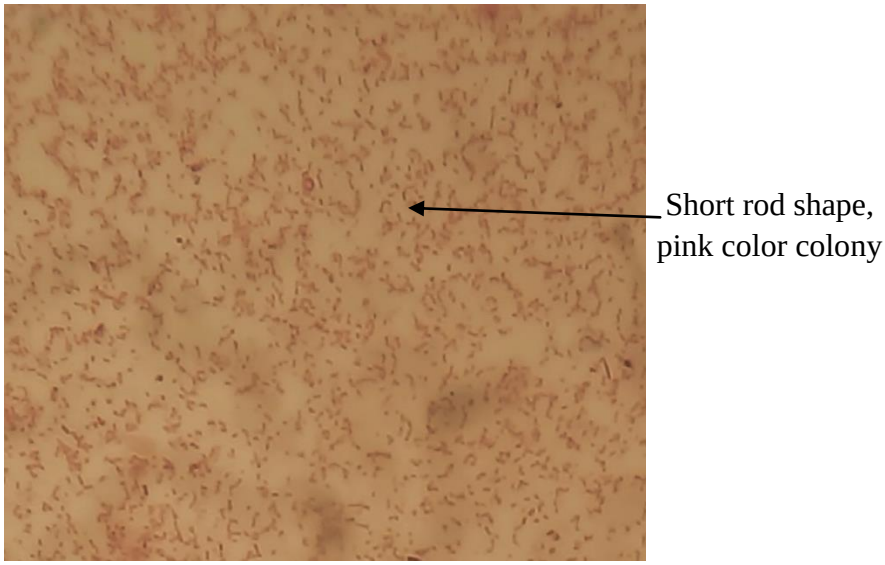

Figure 22: Microscopic observation of *Salmonella* (100X)

4.9 Bio-chemical test to detect *Salmonella sp.*

4.9.1 Catalase test: In catalase test, bubble formation within a few seconds after added 3% H2O2 solution indicated the positive test for *Salmonella*.

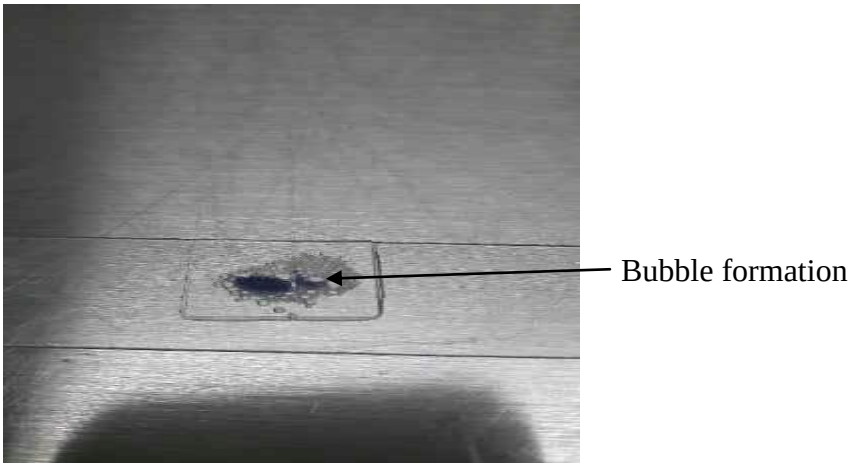

Figure23: Catalase Test (positive)

4.9.2 MR test

After preparation of media, added inoculum into test tube.Then Incubated for 72 hours. After incubation one drop of methyl red solution was added in this test tube. After that red color was indicated positive result & yellow color or no color change indicated negative result.

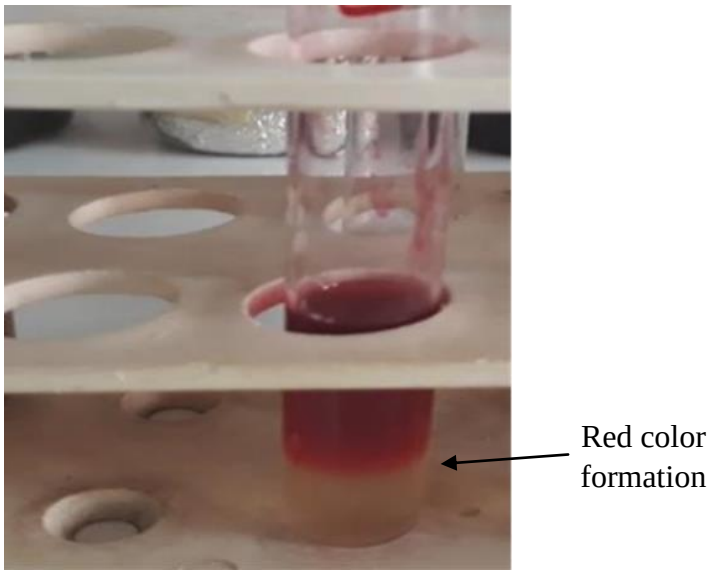

Figure 24: MR Test (positive)

4.9.3 Indole test: After added Kovac's reagent, it forms a ring in between the medium and the ether layer. Development of a brilliant red colored ring indicated indole production. In negative case there is no development of red color (Cheesbrough, 2006)

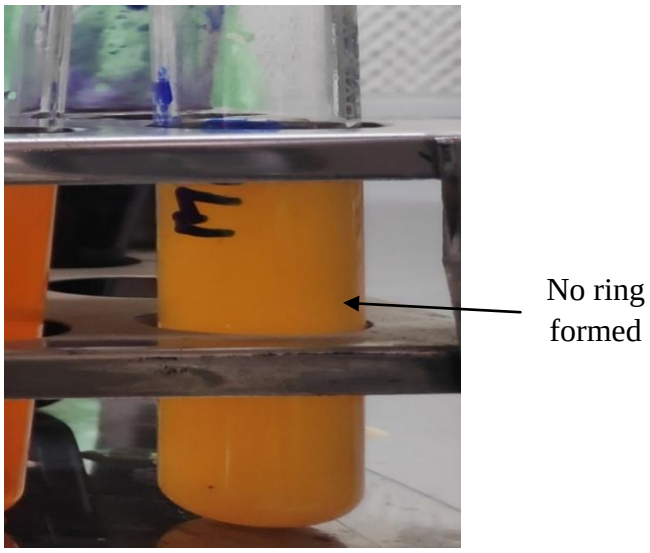

Figure 25: Indole Test (negative)

Salmonella isolated and performed gram's staining and observed under Microscope to identify morphologically. Observed in different Media to identify colony characteristics and observed bio- chemically to identify bio-chemical characteristics.

Table 5: Growth properties, staining properties, microscopic observation and biochemical test of the *Salmonella*

Properties	Observation
Growth Properties	Produce black color colony in SS agar. Produce gray color colony in EMB agar.
Staining Properties	Gram negative
Microscopic Observation	Small rod shape, pink color, arranged single or paired.
Biochemical Test	Bubble formation gives positive indication in Catalase test. Produce red color indicated positive result in MR test. No color change gives negative indication in VP test. No ring formation gives negative indication in Indole test.

Table 6: Prevalence of bacteria in the sample by Total Viable Count (TVC) and Total coliform count (TCC)

Colony count	Total samples	Number of samples containing bacteria	Prevalence (%)
Total Viable Count	60	60	100
Total Coliform Count		20	33.33

By the following (table 3) observation, the TVC was estimated from the colony growth in Nutrient agar media. For this reason, 30-300 colony formed plate was counted, more or less of this amount was discarded. Here observed every sample grew colony on Nutrient agar media, which indicated the presence of bacteria or presence of bacterial contamination in each sample and the prevalence rate is 100%. Another observation by TCC indicated that the 20 samples among 60 samples grew pink color colony in MacConkey agar media and these were considered as the presence of Total coliform bacteria. The prevalence rate of this is 33.33%.

Table 7: Prevalence of bacterial load in the study area

Study area		Total sample	positive		Prevalence (%)
			Salmonella	*E. coli*	
Different Hall	Shiraj-ud-doula Hall	3	0	0	0
	Krishokrotno Sheikh Hasina Hall	3	1	1	66.67
	Kabi kazi Nazrul Islam Hall	3	1	0	33.33
	Begum Sheikh Fazilatunnesa Mujib Hall	3	0	1	33.33
	Sheikh Lutfur Rahman Hall	3	1	0	33.33
	Sheikh Sayera Khatun Hall	3	2	0	66.67
Total	6	18	7		38.89
Different Faculties	Agri. Faculty bhaban	3	1	1	66.67
	Sheikh Kamal Bhaban	3	1	0	33.33
Total	2	6	3		50
Market Areas	Collage gate food shop	3	2	1	100
	Minimarket	3	1	1	66.67
	Food shop campus gate-2	3	1	0	33.33
	Tea stall gate-2	3	1	0	33.33
	Agargaon bazar	3	1	1	66.67
	Paka market	3	1	1	66.67
	Pitha store near gate-1	3	1	1	66.67
	Food shop near gate-1	3	1	0	33.33
	Tea stall near gate-1	3	1	1	66.67
	Jhalmuri shop	3	1	1	66.67
	Fuska shop	3	1	1	66.67
	Different food shop near gate-2	3	1	1	66.67
Total	12	36	22		61.11
TOTAL	20	60	31		51.67

By the Following observation,18 number of drinking water samples were collected from different halls of SAU campus to detect bacterial prevalence. The 3 number of samples showed positive result for *E. coli.,* 5 number of samples showed positive result for *Salmonella* & the prevalence was 38.89%. The prevalence is lower (0%) in Shiraj-ud-doula hall and higher (66.67%) in Krishokrotno Sheikh Hasina Hall, Sheikh Sayera Khatun Hall.

By another observation, from two faculty building 6 number of drinking water samples were collected. Among them a single number of samples showed positive indication for *E. coli.,*3 number of samples showed the positive indication for *Salmonella* and the prevalence is 50%. The prevalence is lower (33.33%) in Sheikh Kamal Bhaban and higher (66.67%) in Agri. Faculty bhaban.

By the observation, 36 samples were collected from different market area in and around the SAU campus area. Among them 11 number of samples showed positive indication for *E. coli,* 14 samples showed positive indication for *Salmonella* and the prevalence is 61.11%. The prevalence is higher (100%) in collage gate food shop and is lower (33.33%) in Tea stall gate-2, Food shop campus gate-2.

Table 8: Prevalence of *E. coli* and *Salmonella* in different areas

Study area	Total sample	Bacteria	Positive sample	Prevalence
SAU halls	18	*E. coli*	2	11.11
		Salmonella	5	27.78
SAU faculties	6	*E. coli*	1	16.67
		Salmonella	2	33.33
Market areas	36	*E. coli*	9	25
		Salmonella	13	36.11

Here is the comparative study among the prevalence of SAU halls, faculties and market samples. The prevalence of E. coli is higher (25%) in market area samples & the prevalence of *Salmonella* is higher (36.11%) in faculty samples.

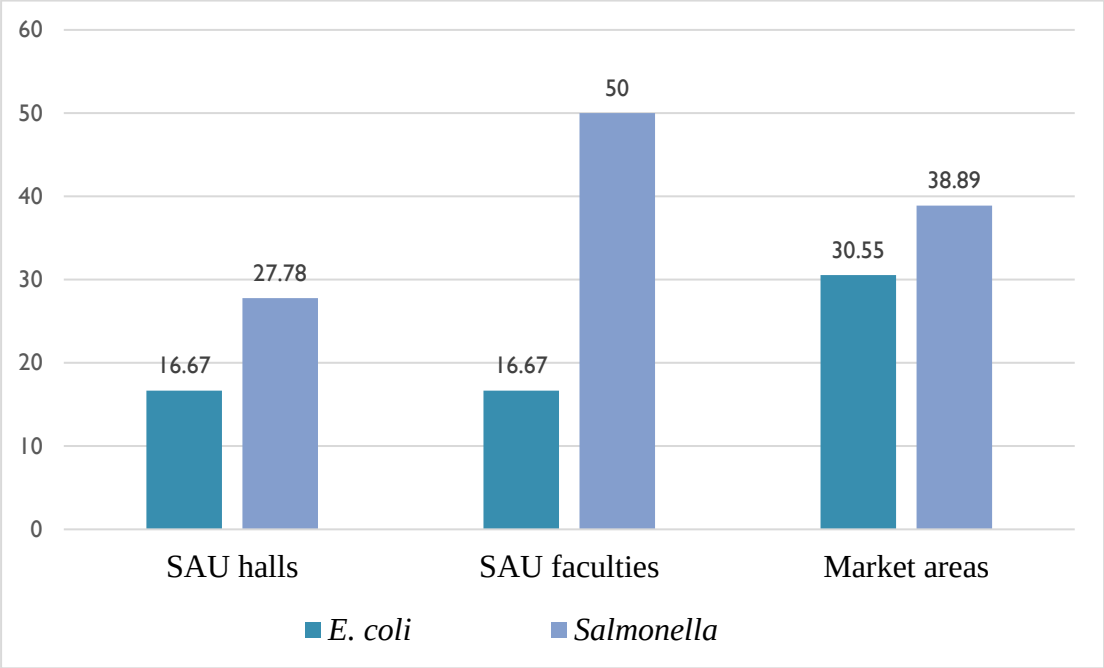

Figure 26: Graphical representation of the prevalence of *E. coli* and *Salmonella* in different areas.

Table 9: Prevalence of bacterial contamination

Total number of samples	Name of the bacteria	Positive samples	Prevalence (%)
60	*Salmonella*	20	33.33
	E. coli	12	20

The 60 samples were collected from different places of SAU Campus and its surrounding areas, among them 15 samples indicated the presence of *E. coli* organism. The prevalence is 20% for *E. coli*.

On the other hand, 22 samples among those 60 samples gives positive result for *Salmonella* bacteria. The prevalence is 33.33% for *Salmonella.*

Table 10: Percentage of individual contamination

Total sample	*Salmonella*	*E. coli*	Mixed
60	17	9	3
prevalence	28.33%	20%	5%

The 60 samples were collected from different places of SAU Campus and its nearby areas, among them 12 samples indicated the individual presence of *E. coli* organism. The prevalence is 15% for *E. coli*, 19 samples indicated the individual presence of *Salmonella* organism and the prevalence is 28.33%, 3 samples indicated the mixed contamination and the prevalence is 5%.

The present study was designed to isolate & identify *E. coli* & *Salmonella spp.* from drinking water sample collected from in & around the SAU campus. For the isolation & identification of the organisms different cultural, morpgological, bio-chemical test was performed.

The results of this investigation indicated that the water samples from Krishokrotno Sheikh Hasina Hall, Begum Sheikh Fazilatunnesa Mujib Hall, Agri. Faculty Bhaban, College gate street food shop, Minimarket, Agargaon bazar, Paka market, Pitha store, Tea stall, Jhalmuri shop, Fuska shop, Different food shop gt-2 contain *E. coli* which is characterized by cultural growth properties (Plate no.- S_2, S_5, S_{11}, S_{15}, S_{23}, S_{26}, S_{29}, S_{35}, S_{41}, S_{44}, S_{46}, S_{54}), morphological properties (Fig-16), bio-chemical analysis(Fig-17, 18, 19).

Another result of this investigation indicated that the water samples from Krishokrotno Sheikh Hasina Hall, Kabi Kazi Nazrul Islam Hall, Sheikh Lutfur Rahman Hall, Sheikh Sayera Khatun Hall, Agri. Faculty Bhaban, Sheikh Kamal Bhaban, College gate street food shop, Minimarket, Agargaon bazar, Paka market, Pitha store, Tea stall , Jhalmuri shop, Fuska shop, Different food shop contain *Salmonella spp.* which is characterized by cultural growth properties (Plate no.- S_1, S_2, S_4, S_{12}, S_{14}, S_{17}, S_{20}, S_{23}, S_{26}, S_{28}, S_{33}, S_{34}, S_{38}, S_{45}, S_{47}, S_{50}, S_{53}, S_{55}, S_{57}, S_{60}) morphological properties (Fig-22), bio-chemical analysis(Fig-23, 24, 25).

According to this present investigation, found 12 samples positive for *E. coli* among 60 water samples. The prevalence rate for E. coli was 20%.

Another observation by (Garba *et al*., 2009) prevalence rate of *E. coli* of this research is 23.3% for tap water sample, which is more or less similar to my observation (20%). Other work by (Sarker et al., 2019) deals with the assessment of bacteriological profile of tube well, jar and pond water to ensure suitability for using and drinking. Among 30 water samples, *E. coli* was present in all pond water samples and 50% tube-well water samples also. The prevalence rate is higher than my observation.

According to my observation, I found 20 samples positive for *Salmonella sp.* Among 60 samples. The prevalence rate was 33.33%. Other observation by (Shahidul et. al.,2014) Prevalence rate of Salmonella was 35% for Dhaka cities restaurant water samples, which is more or less similar to my observation (33.33%).

CHAPTER 5

CONCLUSION

CHAPTER 5

CONCLUSION

This study was performed to detect the quality of drinking water which was supplied to the consumer in and around the SAU campus. This study was performed at the laboratory of microbiology and parasitology department. The duration of this research work was from January,2021 to August, 2021. The sample of drinking water was collected from SAU campus and its nearby areas. Samples were collected for three times in each site. Then physical examination (Color, odor, turbidity, PH, Presence of any foreign particles) was done to know that the samples were suitable for further analysis or not. To detect the bacterial load firstly the sample was incubated in Nutrient broth as a primary inoculum. After incubated overnight in Nutrient broth they produced turbidity which is the indication of bacterial growth. Then ten-fold serial dilution was performed to detect the bacterial load by spreading into media. In this study growth characteristics in different media, staining properties, microscopic vision and biochemical test result was observed for identification of bacteria. The characteristics colonies of *E. coli* were red to bright pink colored in MA agar & greenish red colored with faint metallic sheen in EMB agar. The characteristics colonies of *Salmonella* were black colored in SS agar & gray colored in EMB agar. Both bacteria observed in rod shaped, gram negative, single or paired arranged under microscope. In biochemical test both bacteria showed Catalase test, MR test positive and VP test negative. In case of Indole test *E. coli* showed positive but *Salmonella* showed negative indication. The 60 drinking water samples was collected from 20 different sites of SAU and its nearby areas. In case of different halls sample the prevalence of contaminated water was 38.89%, for faculties this prevalence was 50% and for market areas was 61.11%. The higher prevalence (66.67%) was in Krishokrotno Sheikh Hasina Hall & Sheikh Sayera Khatun Hall. For faculties, the lower (33.33%) prevalence was in Sheikh Kamal Bhaban and higher (66.67%) was in Agriculture Faculty Bhaban. All samples were positive in case of collage gate food shop. The prevalence in this study areas sample for *E. coli & Salmonella* were 20% & 33.33%. But in case of individual bacterial contamination prevalence for *E. coli* was 15%, *Salmonella* was 28.33% and the mixed contamination was 5%. So, direct consumption of this water isn't safe for human and animal. It can cause serious health issue

RECOMMENDATION

This study was conducted to identify the quality of drinking water in the study area. Some samples were found contaminated with *E. coli* and some were with *Salmonella.* It can cause serious health hazard for consumer. To reduce the risk, water should be boiled for 20 minutes prior to drink. So, further analysis should be done to identify the source of contamination, degree of pathogenicity of bacteria and to take precautionary measure.

LIMITATION

Due to Corona virus outbreak in 2019, our research work became so tough to continue from when it declared as a pandemic in all over the world. Strict lockdown was declared in our country, in this crucial situation we couldn't continue our study. The entry of lab wasn't available, it was a huge problem to keep the continuation of our work. After that we didn't have enough facilities in our lab due to lack of instruments and funds.

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APPENDIX

APPENDIX

Composition of different media

1. Nutrient broth

peptic digest of animal tissue	5.000 gm.
Sodium chloride	5.000 gm.
Beef extract	1.500 gm.
Yeast extract	1.500 gm.
Distilled water	1000 ml.
Final pH (at 25ºC)	4 ± 0.2

2. Nutrient Agar

Peptone	5.000 gm.
Sodium chloride	5.000 gm.
HM peptone B#	1.500gm.
Yeast extract	1.500gm.
Agar	15.000gm.

3. MacConkey Agar

Peptones (meat and casein)	3.000 gm
Pancreatic digest of gelatin	17.000 gm
Lactose monohydrate	10.000 gm
Bile salts	1.500 gm
Sodium chloride	5.000 gm
Crystal violet	0.001 gm
Neutral red	0.030 gm
Agar	13.500 gm
pH after sterilization (at 25°C)	7.1±0.2

4. Eosin Methylene Blue Agar

Peptic digest of animal tissue	10.000 gm.
Dipotassium phosphate	2.000 gm.
Lactose	5.000 gm.
Sucrose	5.000 gm.
Eosin – Y	0.400 gm.
Methylene blue	0.065 gm.
Agar	13.500gm.
Final pH (at 25°C)	7.2±0.2

5. *Salmonella-Shigella* agar

Proteose peptone	05.00000 gm.
Lactose	10.00000 gm.
Bile salts mixture	08.50000 gm.
Sodium citrate	08.50000 gm.
Sodium thiosulphate	08.50000 gm.
Ferric citrate	01.00000 gm.
Brilliant green	00.00033 gm.
Neutral red	00.02500 gm.
Agar	13.50000 gm.
Final pH (at 25°C)	7.0±0.2

6. Methyl Red Indicator

Methyl red	0.200 gm
Ethyl alcohol	60.000 ml
Distilled water	

7. Voges–Proskauer (MR-VP) broth

Buffered peptone	7.000 gm.
Dextrose	5.000 gm.
Dipotassium phosphate	5.000 gm.
Final pH (at 25°C)	6.9±0.2

8. Phosphate buffer saline

Sodium chloride	8.0 gm.
Disodium hydrogen phosphate	2.8 gm.
Potassium chloride	0.2 gm.
Potassium hydrogen phosphate	0.2 gm.
Distilled water to make	1000 ml.

Table 11: Bacterial presence among samples

Sample Name	***Salmonella***	***E. coli***	**Both**
S1	+	-	-
S2	-	-	+
S3	-	-	-
S4	+	-	-
S5	-	+	-
S6	-	-	-
S7	-	-	-
S8	-	-	-
S9	-	-	-
S10	-	-	-
S11	-	+	-
S12	+	-	-

S13	-	-	-
S14	+	-	-
S15	-	+	-
S16	-	-	-
S17	+	-	-
S18	-	-	-
S19	-	-	-
S20	+	-	-
S21	-	-	-
S22	-	-	-
S23	-	-	+
S24	-	-	-
S25	-	-	-
S26	-	-	+
S27	-	-	-
S28	+	-	-
S29	-	+	-
S30	-	-	-
S31	-	-	-
S32	-	-	-
S33	+	-	-
S34	+	-	-
S35	-	+	-
S36	-	-	-
S37	-	-	-
S38	+	-	-
S39	-	-	-
S40	-	-	-
S41	-	+	-
S42	-	-	-
S43	-	-	-

S44	-	+	-
S45	+	-	-
S46	-	+	-
S47	+	-	-
S48	-	-	-
S49	-	-	-
S50	+	-	-
S51	-	-	-
S52	-	-	-
S53	+	-	-
S54	-	+	-
S55	+	-	-
S56	-	-	-
S57	+	-	-
S58	-	-	-
S59	-	-	-
S60	+	-	-
Total	17	9	3

Here, (+) means presence and (-) means absence.