DETEMINATION OF MICROBIAL QUALITY OF DRINKING WATER OBTAINED FROM DIFFERENT SOURCES FOR HUMAN, POULTRY AND DAIRY FARM IN DINAJPUR DISTRICT OF BANGLADESH

A THESIS

BY

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REGISTRATION NO. 1705432

SEMESTER: JANUARY-JUNE, 2019

SESSION: 2017

MASTER OF SCIENCE (M.S.)
IN
Veterinary Public Health and Food Hygiene

DEPARTMENT OF MICROBIOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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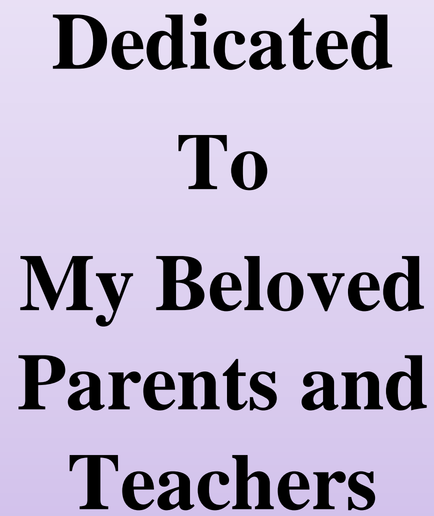
Dedicated
To
My Beloved
Parents and
Teachers

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ABSTRACT

Total ninety (90) samples, thirty 30 from human tubewell water, 30 water samples of dairy farm and 30 water samples of poultry farm were collected and tested for TVC and MPN to to determine the microbial quality of drinking water. The mean HPC of human tubewell water were found 2.55×10^3, 3.11×10^3, 2.85×10^3, 2.88×10^3 and 3.20×10^3 CFU mL^{-1} in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively.The highest HPC was found in Chirirbandar 3.20×10^3 CFU mL^{-1} and lowest found in Sadar 2.55×10^3 CFU $mL^{-1.}$ The MPN values were found 2.16 , 2.16 , 2.50 , 1.83 and 2.33 coliforms/100 ml in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively.The highest MPN value was found in Kaharol 2.50 and lowest were found in sadar 2.16 and Chirirbandar 2.16 coliforms/100 ml .The mean HPC of dairy farm tubewell water were found 2.3×10^3, 3.6×10^3, 2.7×10^3, 3.2×10^3 and 3.4×10^3 CFU mL^{-1} in Sadar, Birgonj, Kaharol ,Birol and Chirirbandar .The highest HPC of dairy farm tubewell water was found in Birgonj 3.6×10^3 CFU mL^{-1} and lowest HPC was found in Sadar 2.3×10^3 CFU $mL^{-1.}$ The MPN values were found 1.66, 2.33, 2.66, 2.00 and 2.66 coliforms/100 ml in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. The highest MPN values were found in Kaharol 2.66 coliforms/100 ml and Chirirbandar 2.66 coliforms /100 ml and lowest was found in sadar 1.66 coliforms/100 ml of water.The mean of HPC of dairy farm manger water were in 4.66×10^7, 2.0×10^7 , 1 2.8×10^7, 2.8×10^7 and 4.2×10^7 CFU mL^{-1} in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively.The highest mean HPC of dairy farm manger water was found in Sadar 4.66×10^7 CFU mL^{-1} and lowest found in Birgonj 2.0×10^7 CFU mL^{-1}.The MPN values were found 14.00, 17.33, 14.00, 16.33 and 15.33 coliforms /100 ml in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. The MPN of dairy farm manger water was highest in Birgonj 17.33 coliforms /100 ml and lowest were found in Sadar 14.00 coliforms /100 ml and Kaharol 14.00 coliforms/100 ml.The geometric mean of HPC of Poultry farm (Tubewel/Machine) water were Sadar 2.8×10^3 CFU mL^{-1}, Birgonj 2.67×10^3 CFU mL^{-1}, Kaharol 3.0×10^3 CFU mL^{-1}, Birol 2.57×10^3 CFU mL^{-1} and Chirirbandar 3.0×10^3 CFU mL^{-1}.In this study, it was found that HPC of Poultry farm tubewell water was highest in Kaharol 3.0×10^3 CFU mL^{-1} and lowest in Birol 2.57×10^3 CFU mL^{-1}.The MPN values of Poultry farm tubewell water were found 2.66, 2.00, 2.33, 1.66 and 2.00 coliforms/100 ml of drinking water in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. The MPN of Poultry farm tubewell water was highest in Sadar 2.66 coliforms/100 ml and lowest in Birol 1.66 coliforms/100 ml.The mean of HPC of Poultry farm waterer water were found 4.2×10^7, 2.00×10^7, 3.25×10^7, 1.00×10^7 and 2.00×10^7 CFU mL^{-1} in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. Highest HPC of Poultry farm waterer water was found in Sadar 4.2×10^7 CFU $mL^{-1}$and lowest in Birol 1.00×10^7 CFU mL^{-1}. The MPN values of poultry waterer water were 12.33, 9.66, 10.33, 13.33 and 12.33 coliforms/100 ml in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. The highest MPN value was found in Birol 13.33 coliforms/100 ml and lowest were found in Birol 9.66 coliforms/100 ml. From ninety water samples seven bacteria were isolated among them the most frequent isolate was *Escherichia coli* 82(15.95%) followed by *Salmonella* spp 81(15.75%), *Shigella* spp 80(15.56%), *Klebsiella* spp 79(15.36%), *Vibrio* spp 73(14.20%), *Pseudomonas* spp 60(11.67%) and *Staphylococcus* spp 59(11.47%). The antimicrobial susceptibility pattern showed that the isolates were highly resistant to ampicillin, amoxicillin, erythromycin and chloramphenicol and susceptible to gentamicin, azithromycin, colistin, ceftriaxone, levofloxacin and ciprofloxacin. Out of seven bacteria two bacteria *(Escherichia coli* and *Vibrio* spp) are subjected to amplified by using 16S rRNA gene based PCR.

Key words: HPC: Heterotrophic Plate Count; TVC: Total Viable Count; MPN: Most Probable Number

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LIST OF ABBREVIATIONS

-	:	Negative
%	:	Percentage
+	:	Positive
µg	:	Microgram
^{0}C	:	Degree of Celsius
Assist	:	Assistant
CIP	:	Ciprofloxacin
CEF	:	Cefixime
E	:	Erythromycin
E. coli	:	*Escherichia Coli*
e.g	:	Example
EMB	:	Eosin Methylene Blue
et al.	:	Associated
Etc	:	Etcetera
EUCAST	:	European Committee on Antimicrobial Susceptibility Testing
GEN	:	Gentamycin
Gm	:	Gram
H_2O_2	:	Hydrogen peroxide
H_2S	:	Hydrogen sulphide
Hrs	:	Hours
HSTU	:	Hajee Mohammad Danesh Science and Technology University
i.e.	:	That is
Ib	:	Pound
Kg	:	Kilogram
KOH	:	Potassium hydroxide
M.S	:	Master of Science
MC	:	MacConkey Agar
MDR	:	Multidrug resistant
MI	:	Milliliter
Min	:	Minute
MIU	:	Motility Indole Urease
MR	:	Methyl Red
MSA	:	Mannitol Salt Agar
NA	:	Nutrient Agar
NB	:	Nutrient Broth
No.	:	Number
SL	:	Serial number
Spp.	:	Species
SSA	:	Salmonella Shigella Agar
TSI	:	Triple Sugar Iron
v/v	:	Volume by volume
VP	:	Voges-Proskauer
HPC	:	Heterotrophic Plate Count
TVC	:	Total Viable Count
MPN	:	Most Probable Number

CHAPTER 1

INTRODUCTION

Water is essential for all living beings. Without water, life cannot be sustained beyond a fewdays. Almost all of the biochemical reactions occur in the presence of water. Drinking safe water has several health benefits like regulating appetite, increase metabolism, boost energy levels and help reduce blood pressure. Water also helps to maintain the internal body temperature and fluid balance (Hameed *et al.,* 2015). The quality of drinking water is closely associated with human health, and providing safe drinking water is one of important public health priorities. Peoples obtain their drinking water from surface and underground sources. However both surface and ground water sources could become contaminated by biological and chemical pollutants arising from different sources. Surface water sources are lakes, river and stream. The qualities of surface water rapidly alter as a response to alteration in the surrounding environment. Other contamination sources of surface water are domestic waste water and sewage, storm water runoff, cattle feedlots runoff etc (Geldreich, 1990).

Water is essential for all usual domestic purposes, and is an fundamental ingredient of household, economic and agricultural activities (Wu *et al.* 2017), while insufficient water supplies prevent good sanitation and hygiene practices (Dey *et al.* 2015). Pure drinking water is a basic need for the people of all over the world. A huge percentage of people of the world are deprived from the pure drinking water including Bangladesh. Most of the rural and urban people of Bangladesh are deprived from availability of pure drinking water due to lack of public awareness, proper sanitation facilities, modern technology and water lifting in unhygienic condition etc. Most of the people are depended on untreated groundwater and tubewells. But in this country, 11% of all deaths are estimated to be caused by diarrheal disease (Streatfield *et al.,* 2001).

About 1/3 of the total world population use ground water for drinking purpose (Nickson *et al.,* 2005). Safe drinking water is a basic need for good health and it is also a basic right of humans. Fresh water is already a limiting resource in many parts of the world. In the next century, it will become even more limiting due to increased population, urbanization and climate change (Jackson *et al.,* 2001). Pure drinking water and hygienic sanitation are fundamentals for health, but these associations achieve greater importance when large numbers of displaced people seek shelter in overcrowded settlements where

there are no established facilities to encourage hygiene and other barriers to prevent the transmission of disease. The greatest risk from microbes in water is accompanying with consumption of drinking water that is contaminated with human and animal excreta (WHO., 2006). About 1.1 billion people globally drink unsafe water (McMichael *et al.,* 2006).

Public and environmental health defense requires safe drinking water.Bacteriological quality of drinking water is primarily determined by using indicator organisms, whose presence indicates fecal contamination. Higher the level of indicator bacteria, higher the level of fecal contamination and greater risk of constricting disease (Hannan *et al.,* 2010). A large number of infectious diseases are spread primarily through water supplies contaminated with human and animal excreta predominantly faeces (WHO., 1993). Most common diseases caused by polluted water are diarrhoea, dysentery, cholera, typhoid, infectious hepatitis, pyogenic infections, gastroenteritis, eye, ear and skin infections and urinary tract infections etc (Bharti *et al.,* 2003). Water correlated diseases are the major cause of morbidity and mortality worldwide. Estimated 80 percent of all diseases and over one third of deaths in developing countries are caused by the drinking of contaminated water. Among these diarrheal diseases are estimated to cause 1.8million deaths each year (Zuthi *et al.,* 2009).

At least 25 million deaths each year are liable on these water-related diseases,including nearly two-third of the moralities of children under five years old. The main source of these pathogens is from untreated or inappropriately treated human waste. Drinking water supplies have a long history of being infected by a wide spectrum of microbes. Therefore, the prime goal of water quality management from health perspective is to ensure that consumers are not exposed to pathogens that cause disease. Protection of water sources and treatment of water supplies have greatly reduced the incidence of these diseases in developed countries. Therefore, testing the source of water is necessary, especially when there is no water treatment. This is useful as result of the catastrophe of treatment process or as a part of an investigation of serious water-borne disease outbreak (Howell., 2001).

Pathogens that cause diarrheal diseases are being linked with contaminated water consumption, such pathogens are the main cause of gastrointestinal infections. The childhood mortality rate due to diarrheal diseases is 2.5 million each year (Muhammad *et*

al., 2012). Each year approximately five million children die due to the use of dangerous water (Shar *et al.,* 2010). Water pollution is the specific impairment of water quality by agricultural, domestic or industrial wastes to a degree that has an contrary effect upon any beneficial use of water yet that does not necessarily create an actual hazard to public health. Due to urbanization and industrialization, waste water that is being discharged into natural water bodies results in serious ground water contamination (Awan *et al.,* 2002).

The decreasing availability of water supplies is one of the most vital environmental issues faced by various countries at the present time. It has been estimated that approximately two-third of nations world-wide will experience water stress by year 2025 (Sim *et al.,* 1987). According to United Nations, subsidiary organizations and other international organizations, 1.1 billion people are without a sufficient access to water, and 2.4 billion people have to live without suitable sanitary water. Under current trends, the prognosis is that about 30 billion people of a population of 8.5 billion will suffer from water shortage by 2025 (Hadji., 2004).

In the agricultural sector water is central to many activities; from crop, poultry and dairy production to animal husbandry, and is also used in domestic activities. Faecally derived pathogens such as *Escherichia coli*, reduce the water quality, which when used in a dairy parlour could reduce milk quality (Esterhuizen *et al.,* 2014). Organisms found in polluted water typically lead to gastrointestinal symptoms, such as nausea, diarrhoea and stomach cramps. The most common organisms include *non-typhoidal Salmonella, Giardia, Shigella, Campylobacter*, *Microsporidium* and *Cryptosporidium* (Lund and O'Brien, 2011). The drinking water plays an important role in the transmission of many pathogenic agents among poultry. Microbial contamination above the acceptable levels in drinking water can directly affect health and performance. Fecal contaminated well water is a source of coliforms such as *E. coli* that may cause *colibacillosis* in poultry flocks. There have been many reports about water contamination with the main poultry pathogens such as *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* (Amaral *et al.,* 2004). Animal manures are known to contain pathogenic bacteria, viruses and parasites and pose a significant threat to human health through the consumption of water polluted by these organisms.

Safe drinking water is a basic need for good health and it is also a basic right of humans. Fresh water is already a limiting resource in many parts of the world. In the next century, it will become even more limiting due to increased population, urbanization and climate change (Jackson *et al.,* 2001). Important factors to prevent waterborne diseases in livestock and poultry production are the protection of supply sources, water disinfection and the quality control of microbiological, chemical and physical characteristics. In our country dairy and poultry owner have little knowledge of the effect of poor farm hygiene practices resulting outbreak of infectious diseases occur in the farm. Poor personal hygiene and minimal management of drinking water quality may enhance the chance of disease occurrence in human. Therefore assessment of water quality can provide insight into the sustainability of water supply. So now a day's improvement to water quality has taken on a greater urgency in our country. Microbiological examination of water should routinely be carried out to control the quality of drinking water. Although substantial amount of work has been carried out in Bangladesh, unfortunately a little information is available.

Bacteriological examination of water samples are usually undertaken to approximate the water quality. Most of the waterborne disease is related to faecal pollution of water sources. Therefore water microbiology is largely based on the need to identify indicators of faecal pollution such as *Escherichia coli* (Barrell *et al.,* 2000). Safety of drinking water remains an important public health concern particularly in emergency situations (Ferretti *et al.,* 2010).

Considering the above circumstances the study was conducted to assess the microbiological contamination of drinking water in Dinajpur District of Bangladesh. Therefore, this research will play a great significance role in public health concerns in developing countries like Bangladesh.

So the study was conducted with the following specific objectives

- To enumerate total bacterial count
- To isolate and identify bacterial pathogens from collected water sample by using morphological, cultural and biochemical technique
- To characterise identified isolates (*Escherichia coli* and *Vibrio* spp) by using PCR

CHAPTER 3

REVIEW OF LITERATURE

2.1 To isolate and identify bacterial pathogens from collected water sample by using morphological, cultural and biochemical technique

Sanganyado *et al.,* (2019) observed the occurrence of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in treated and untreated drinking water has been a growing interest in the recent years, ARB and RGs pose a public health concern when they transfer antibiotic resistance (AR) to human pathogens. However, it is still unclear whether the presence of environmental ARB and ARGs in source water, drinking water treatment plants, and drinking water distribution systems have any significant impact on human exposure to pathogenic ARB. In this review, we critically examine the occurrence of AR in groundwater, surface water, and treated distributed water. This offered a new perspective on the human health threat posed by AR in drinking water and helped in crafting a strategy for monitoring AR effectively. Using existing data on removal of ARB and ARGs in drinking water treatment plants, presence and proliferation of AR in drinking water distribution systems, and mechanisms and pathways of AR transfer in drinking water treatment plants, they concluded that combining UV-irradiation with advanced oxidative processes (such as UV/chlorine, UV/H2O2, and H2O2/UV/TiO2) may enhance the removal of ARB and ARGs, while disinfection may promote horizontal gene transfer from environmental ARB to pathogens.

Goel *et al.,* (2019) conducted a stratified random cross-sectional survey of 484 households to assess microbial contamination of deep tubewell water at source and at point of use (POU) compared to shallow tubewell water using the Compartment Bag Test. They measured storage time, distance, travel time and ownership status among both sets of users to assess deep tubewell efficacy and under what conditions they offer poorer or better water quality. There was no significant difference in microbial contamination between shallow and deep tubewells at source. The presence of POU water microbial contamination in storage containers in deep tubewell households was 1.11 times the prevalence in shallow tubewell storage containers (95% CI = 0.97–1.27). Deep tubewell users stored water longer and walked significantly farther to obtain water compared to shallow tubewell users. Among deep tubewell households, those residing farther away from the source were 1.24 times as likely to drink contaminated water from storage

containers compared to those located nearby (95% CI = 1.04–1.48). Our findings suggest that deep tubewells have comparable water quality to shallow tubewells at source, but increasing distance from the household exacerbates risk of microbial contamination at POU.

Champa ***et al.,*** **(2018)** collected 20 tap water samples and the methods of heterotrophic plate count (HPC) and total coliform count (TCC) were applied. Moreover, isolated E. coli from tap water samples were characterized by using biochemical test, molecular method and antimicrobial susceptibility tests. HPC was highest in market tap water collected from Sarishabari of Jamalpur district and TCC was highest in market tap water collected from Durgapur of Netrokona district. The geometric mean of HPC of Jamalpur, Tangail, Kishoreganj and Netrokona districts water were 4.2x107, 2.7x107, 2.4x107 and 4.4x107 CFU/ml respectively. Out of 13 isolates of E. coli, 13 isolates were amplified by using 16S rRNA gene based PCR. The antimicrobial susceptibility pattern showed that the isolates were highly resistant to amoxicillin. Most of the E. coli isolates were susceptible to tetracycline, azithromycin, streptomycin, norfloxacin and ciprofloxacin. Furthermore, a few E. coli isolates were intermediate resistant to erythromycin and gentamycin. Moreover, out of 13 E. coli isolates 2 (15.4%) isolates were detected as multidrug resistant. This study indicated the presence of multidrug resistant E. coli isolates in tap water in Jamalpur, Tangail, Kishoreganj and Netrokona districts that warrant particular attention.

Jain ***et al.,*** **(2018)** analysed the physicochemical and bacteriological examination of ground water (Tap water) seasonally (rainy, winter and summer seasons) at selected regions of Mhow Tehsil area (Sangi Street, Raj Mohalla, Kali Mata Mandir area, Cantonment Board area and Main Street) for two years, during 2011-2013. During physicochemical examination, Water Colour, Temperature (°C), pH values, Total hardness (mg/lit), Specific conductivity (µmhos/cm), Total alkalinity (mg/lit), TDS (mg/lit), Chloride (mg/lit), Fluoride (mg/lit), Nitrate (mg/lit), Phosphate (mg/lit), Sulphate (mg/lit) and BOD (mg/lit), DO (mg/lit) and COD (mg/lit) values have been analyzed however, the total coliforms (MPN/100ml) and faecal coliforms (MPN/100ml) were also estimated during bacteriological examination. Continuous monitoring and environment management programs should be run properly to manage the elements in limit range which is necessary to control drinking water pollution.

Kormoker ***et al.,*** **(2017)** collected a total of nine samples, one from each ward to the user's house and analyzed for E. coli which represented the quality of the piped water. A total of three water samples were collected from each of the ward for microbiological (FC) quality analysis. Millipore microbiological field testing kits was used for E. coli testing, DR 2800 HACH spectrophotometer was used for determination of manganese, iron, and nitrate and Wegtech Digital Arsenator was used for arsenic determination. Physical parameters were tested by HACH potable instruments. The light brown yellowish color created an aesthetic problem among the water users of Barguna Pourashava. In addition, the fecal contamination rate was very high. The findings of E. coli analysis of Barguna Pourashava supply water indicated that 11% have intermediate risk, 37% have high risk and 52% have very high risk considering the health. The water of the production wells of Faridpur Pourashava has high concentration of the arsenic, iron and E. coli. The household's storage water sample analysis result indicated that 48%, 44% and 7% have very high risk, high risk and intermediate risk from the health point of view.

Ravenscroft ***et al., (*****2017)** detected faecal coliforms (FC) in 3.3–23.3% of samples at four sites. They differentiate a near-field, characterised by high concentrations and frequent, persistent and contiguous contamination in all directions, and a far-field characterised by rare, impersistent, discontinuous low-level detections in variable directions. Far-field FC concentrations at four sites exceeded 0 and 10 cfu/100 ml in 2.4–9.6% and 0.2–2.3% of sampling events respectively. The lesser contamination of *in-situ* groundwater compared to water at the point-of-collection from domestic wells, which itself is less contaminated than at the point-of-consumption, demonstrates the importance of recontamination in the well-pump system. Applying a hypothetical dose-response model suggests that 1–2% of the diarrhoeal disease burden from drinking water is derived from the aquifer, 29% from the well-pump system, and 70% from post-collection handling.

Luby ***et al.,*** **(2015)** used a prospective, longitudinal cohort enrolled as part of a program evaluation to assess the relationship between drinking water microbiological quality and child diarrhea. Laboratory technicians measured the concentration of Escherichia coli in the water samples using membrane filtration. Of drinking water samples, 59% (2,273/3,833) were contaminated with E. coli. Of 12,192 monthly follow-up visits over 2 years, mothers reported that their child had diarrhea in the preceding 2 days in 1,156

(9.5%) visits. In a multivariable general linear model, the log10 of E. coli contamination of the preceding drinking water sample was associated with an increased prevalence of child diarrhea (prevalence ratio = 1.14, 95% CI = 1.05, 1.23). These data provide further evidence of the health benefits of improved microbiological quality of drinking water.

Shahid *et al.,* (2015) investigated the concentration of various pollutants in water samples collected from student hostels of the University of the Punjab in Lahore, Pakistan, and the potential risk of these pollutants for health. A total of 18 samples from 12 different locations were assessed for physical, biological, and chemical contaminants using standard methods. The concentrations of arsenic (As) and coliform bacteria were above the international standards given by the World Health Organization (WHO). The range of as concentration was from 24.92 to 32.72 μg·L-1. Similarly, the two water samples showed bacterial contamination 38 MPN/100 mL and 21 MPN/100 mL exceeding the standard value set by WHO (0 MPN/100 mL). We conclude that drinking water quality was poor, as is evident from the high concentration of As. Urgent measures are required to prevent such contamination and regular monitoring of drinking water quality in the study area.

Adzitey *et al.,* (2015) determined the occurrence of E. coli in drinking water sources used by humans and farm animals in Nyankpala community of Ghana. Isolation of E. coli was done using a slightly modified procedure in the US Food and Drug Administration-Bacteriological Analysis Manual (FDA-BAM). A total of 200 water samples collected from six different water sources viz. sachet water (four different brands), tap water, well water, dam water, bottle water and water from the drinking troughs (drinkers) of farm animals were analyzed. The average occurrence of E. coli in the different water samples was 58 (29%). The highest occurrence of E. coli was in well water 100% (20/20), followed by water from drinkers 80% (12/15), dam water 65% (13/20), rain water 50% (10/20) and tap water 10% (3/25). All sachet (0/80) and bottle water (0/20) samples were negative for E. coli. The number of well water samples positive for E. coli was significantly higher ($p<0.01$) than that of dam water, sachet water, rain water and tap water. This work indicated that some drinking water samples (well, drinkers, dam, rain water and tap water) in the Nyankpala Community of Ghana are contaminated with E. coli and thus humans and farm animals are at risk of foodborne infections from drinking water from such sources.

Machado *et al., (*2014) measured the water quality and the prevalence of antibiotic resistance of heterotrophic culturable bacteria were characterized seasonally in wells that serve the population of Guinea-Bissau (West Africa) as the sole source of water for drinking and other domestic proposes. The results revealed that well water was unfit for human consumption independently of the season, owing to high acidity and heavy fecal contamination. Moreover, potentially pathogenic bacteria, which showed resistance to the most prescribed antibiotics in Guinea-Bissau, were isolated from well water, posing an additional health risk. Our results suggest that well water not only fosters the transmission of potential pathogenic bacteria, but also represents an important reservoir for the proliferation of antibiotic resistant bacteria, that can aggravate the potential to cause disease in a very vulnerable population that has no other alternative but to consume such water.

Chowdhury *et al.,* (2014) represented the water quality used in hills tracts in Chittagong in Bangladesh from microbiological point of view. 29 water samples from different hills tract areas and sources which are consumed by the people of hill tracts analyzed where every water sample was unacceptable for consumption according local and international pure drinking water guidelines for microbiology. Among the 29 samples, all the samples were tested for 5 microbial parameters. The range for Total Plate Count (TPC) is too numerous to count (TNTC) in every sample, 16 samples were contaminated by total coliforms, 4 samples were contaminated by *E. coli,* and 6 samples were by with *K. pneumoniae* and *P. aeruginosa*. Ringwells are highly contaminated comparatively other sources of water. Lacks of sanitation, hygiene, awareness about health, education and unavailability of water treatment lead the unwanted contamination in these sources of drinking water.

Farzana *et al.,* (2014) collected total of fifty two domestic tap water samples were c from different areas of Karachi, between May to June 2011 and analyzed for bacterial presence based on biochemical testing. The results revealed a high prevalence of Bacillus spp. (86.84%), followed by Pseudomonas spp. (57.14%), *Citrobacter* spp. (14.28%) *Serratia* spp., *Enterobacteriaceae* species (14.28%), *Corneybacterium* (10.52%), and *Acinetobacter* spp. (2.63%). These findings disclose bacterial contamination in drinking water supplies, many of which are pathogenic and can produce serious as well as life-threatening infections. Future studies will determine whether bacterial contamination of

drinking water occurred post-source contamination. It is recommended that household water treatment interventions should be introduced to improve water qualit

Acharjee *et al., (*2014) assessed the pathogenic prevalence in municipal water supplied across Mirpur zone, Dhaka, and its subsequent health impact on the local community. Elevated numbers of pathogenic bacteria including *Aeromonas* spp, *Shigella* spp, *Staphylococcus* spp, and *Salmonella* spp. were found in the consumer points ($n = 30$) compared to that of the supply points ($n = 10$). Additionally, proliferation of fecal coliforms, *Escherichia coli* and *Klebsiella* spp. was monitored among the consumer points and not in the supply points. Drug resistance was scored against ampicillin (10 μg), ciprofloxacin (5 μg), ceftriazone (30 μg), penicillin (10 μg), nalidixic acid (30 μg), and vancomycin (30 μg). Hence, the municipal water of the study area was microbiologically unsafe, and the propagation of drug resistant strains was assumed to escalate the public health threat. A survey on public opinions was also conducted to evidently chalk out the impact of municipal water on the specific community studied.

Ali *et al.,* (2013) analyzed the bacterial quality of drinking water by Total Plate Count (TPC), Total Coliform Bacteria (TCB), Total fecal Coliform Bacteria (TFCB) and Escherichia coli O157:H7. Bacteriological analysis of tube well drinking water results revealed that highest TPC values 576 cfu/ml and the lowest were 76 cfu/ml, TCB were found in the range 23-<1.1MPN/100ml, TFCB were found in the range 16-<1.1MPN/100ml and E. coli O157:H7 were present in four localities water samples. The Hand pump water samples showed that TPC were in the range 234-32cfu/ml, TCB were found in the range 23-<1.1MPN/100ml, highest TFCB (MPN/100ml) values 16 and lowest values <1.1 and 50% samples were contaminated with E. coli O157:H7. Open well water samples analysis data indicated that 80% samples were unfit due to higher TPC values for human consumption according to WHO standards, 70% samples were unfit due to higher (TCB) and TFCB than permissible limits and 70% samples were found E. coli O157:H7.

Akbar *et al.,* (2013) detected thermo-tolerant fecal coliform (Escherichia coli) by using Portable water testing kit (Oxfam-Del-Agua). A total (n=254) number of drinking water samples were examined for the presence of fecal coliform. It was found that, 68.5% (174 out of 254) of the overall samples tested were contaminated with Escherichia coli. The 52% (40 out of 77) of the water samples at source level, whereas 69% (58 out of 84) of

water sample collected from system and 71% (66 out of 93) at household level were found contaminated with Escherichia coli. It was concluded that unprotected drinking water sources, improper management of waste, vulnerable sewage and ignorance regarding health and hygiene are the main reason of water contamination in the area.

Shah ***et al.,*** **(2013)** collected 33 water samples from different locations of Swabi and from different sources such as lake water, tap water, and spring.0 for bacteriological test, to find out which bacteria are present in it. Different tests have been done for different bacteria i.e. coliform, Fecal coliform, and *Escherichia coli* (*E. coli*). Out of 33, 22 samples were fit for drinking, and the remaining 11 samples were not fit for drinking which contained tap water and lake water. This study revealed that in most of the villages in Swabi, the water quality is good than other nearby districts and major cities which reflects the awareness of the local population about how to maintain, store and use the water for drinking purposes. Other reason could be the less number of the waste water sources in Swabi in comparison to the well-populated cities.

Khan ***et al.,*** **(2013)** investigated the concentrations of various pollutants in drinking water and health risk in Charsadda district, Khyber Pakhtunkhwa, Pakistan. Water samples were collected from dug-wells, tube-wells and hand pumps which were the most common sources of drinking water and analyzed coliform bacteria using standard methods. The coliform bacterial contamination (2–5 MPN 100 mL^{-1}) was also found in some sources of water, confirming the bacterial contamination of drinking water. Water contamination with coliform bacteria was the main source of waterborne diseases like gastroenteritis, dysentery, diarrhea and viral hepatitis as complained by most of the respondents during questionnaire survey.

Datta ***et al.,*** **(2013)** investigated the presence pathogenic bacteria and their antimicrobial resistance pattern of groundwater of Sitakunda, Chittagong. In this study bacterial contamination in tube well water has been analyzed in terms of coliform, fecal coliform and vibrio. Qualitative analysis of bacteria is performed by spread plate technique and antibiotic susceptibility test is done by disk diffusion method. In bacteriological analysis total coliform, total fecal coliform, and total vibrio spp. count are ranged from 0.65×102 to 2.0 ×102 cfu/ml, 0.10 ×102 to 2.20 ×102cfu/ml and 0.0 to 0.55×102cfu/ml, respectively. All of the coliform isolates are resistant to ampicillin, all fecal coliform isolates to penicillin and sulphamethoxazole and vibrio spp. are resistance to

erythromycin and azithromycine. The coliform isolates also display multidrug resistance. The resulting contamination of tube well water with pathogen and their resistivity to antibiotic is an alarming threat to public health, so tube well water of Sitakunda, Chittagong must be treated prior to drinks.

Mahbub *et al.,* (2011) assessed the microbiological quality of Dhaka WASA drinking water. A total of 45 samples were collected from different outlets of WASA water supply chain. Among the these samples 29 samples were collected from house tap, 5 samples from street pipe line tap and 11 samples from WASA source pump. The results of the Total Viable Count (TVC) showed that 62 % samples of house tap water, 60 % pipeline water and 45.45 % WASA pump water were exceeded the BDS standard (1240:2001) and WHO Guideline for drinking. The highest count was 2 × 106 cfu/ml in the house tap water of Gandaria. Total coliform and E. coli count ranged from <1.8 (MPN) /100 ml to >1600 (MPN)/100 ml. Among all the tested samples, 57.78 % water samples were positive for coliform and 51.11 % samples were positive for E. coli bacteria. Out of twenty three E. coli isolates, 8 isolates were subjected to biochemical and microscopic examination for confirmation. All 8 isolates were detected as E. coli based on biochemical parameters. The antibiotic sensitivity pattern of those isolates was determined. Most of them were found resistant to Ampicillin, Amoxicillin, kanamycin, Penicillin, Sulphomethoxazole antibiotics. Nearly all of them were found sensitive to Gentamycin and Nalidixic acid. The samples collected from different house tap water and road side tap water were more contaminated than WASA source pump water.

Ali *et al.,* (2011) evaluated the quality of drinking water in Khyber Agency valley, which has always been crucial with reference to public health importance. Quality assessment of drinking water were carried out by determining, total plate count, total coliform bacteria, total fecal coliform, *E. coli* and *Staphylococcus aureus*. The total plate count (TPC) was found in the range of 3600-190 CFU/ml which indicated that none of the samples were found in drinking water according to the WHO standards (100 CFU/ml).

Hannan *et al.,* (2010) collected a volume of 100 ml water was filtered under the vacuum pressure through Millipore membrane filters. After filtration, membrane filters were placed on CHROM agar and incubated at 35C for 24 hr. Escherichia coli appeared as blue coloured colonies while coliforms yielded colonies of pink colour. Escherichia coli were further identified by API 20E and confirmed by Eijkman test. Results: *Escherichia*

coli was grown from 42% samples (all Eijkman positive). Coliform organisms were grown from 54% specimens. Conclusion: It was alarming that 59% of drinking water was unsatisfactory for human consumption.

Anwar *et al.,* (2010) collected a total of 530 water samples were collected from different localities of whole of the Lahore city. These represented areas with different socio-economic conditions. The samples were collected in sterilized containers and brought to the laboratory within two hours of collection. All the samples were tested for contamination with bacteria using multiple tube method to determine most probable number of total coliforms and faecal coliforms using standard procedure. Results: Among 530 water samples, 197 samples (37.2%) were positive for bacterial contamination. It was observed that bacterial contamination was maximum in areas with low SEC (43.6%), followed by intermediate SEC (36.5%) and high SEC (22.9%). The difference was found to be statistically significant ($p<0.15$) between areas with High and Low SEC while it was non-significant ($p>0.5$) between areas with Low and Intermediate SEC.

Islam *et al.,* (2010) examined microorganisms in tap water comprised Escherichia coli spp. (60%), *Klebsiella* spp. (40%), *Enterobacter* spp. (20%), *Pseudomonas* spp. (70%), *Proteus* spp. (10%), *Staphylococcus* spp. (40%) and *Salmonella* spp. (0%). Furthermore, there was no correlation between faecal coliform and the presence of Salmonella species. Results obtained from this investigation revealed that municipal tap water of Dhaka city was contaminated with a number of enteric bacteria such as *E. coli.* This organism was considered as a good bioindicator model for surveillance studies of antimicrobial resistance. So, only antibiotic resistance pattern of *E. coli* was determined. A total of 10 *E. coli* isolates were used for the sensitivity test. All the isolates were totally resistant to Rifampin and Bacitracin (100%). Most of the isolates were found highly resistant to Tetracycline (90%) and Erythromycin (90%), moderately resistant to Amoxicillin (70%), Streptomycin (70%) and Novobiocin (60%). On the other hand, the isolates were totally sensitive to Gentamycin (100%) and Kanamycin (90%) and highly sensitive to Chloramphenicol (80%).

Bassam *et al.,* (2010) examined water samples for the presence of potential pathogens and for their resistance to antibiotics by the disc diffusion method. Sabil water has a high bacterial contamination. Coliforms (39.71%), E. coli (13.24%) and other pathogenic

bacteria were widely represented in the investigated Sabil water. In a first step to screen the waters for potentially pathogenic properties, 163 (47.8%) of the isolates showed α- or γ-haemolysis on human blood agar media. Among the haemolytic isolates, 45.1% were resistant to clindamycin and 52.3% to ampicillin. The most commonly isolated genera with these potentially pathogenic features were Bacillus spp., Streptococcus spp. Staphylococcus spp., Corynebacterium spp., E.coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeuginosa, Aeromonas veronii, Acinetobacter spp. and Aeromonas hydrophila. While the most frequently isolated fungal species were *Alternaria* spp*.,* *Aspergillus* spp*.,* *Cladosporium* spp*.,* *Epidermophtton* spp*.,* *Microsporum* spp*.,* *Penicillium* spp.

Zuthi *et al.,* (2009) identified water contamination problems, water samples from different locations of the four routes of the distribution network of CWASA were analyzed in this study. The study revealed that most of the important water quality parameters except BOD_5 were in the permissible limit. More than 95% of the collected water samples had BOD_5 greater than 0.20ppm with maximum of those found 5.2ppm at a house connection near Polytechnic College. Microbial water quality parameters examined at some selected locations showed the presence of pathogenic organisms in water exceeding the permissible limit. Few sources of contamination along the CWASA's distribution network have been identified. In order to avoid water contamination problems, awareness raising and regular monitoring of water distribution network have been suggested in this study.

Warner *et al.,* (2008) most problematic were total coliform and Escherichia coli bacteria, which were present in 94 and 72% of all the water samples, respectively being the shallowest, were the most contaminated by bacteria and nitrate; deep-aquifer tube wells were the most contaminated by arsenic. Whereas E. coli concentrations decreased with depth, iron and ammonia concentrations increased with depth. These relationships account for people choosing to drink water with higher levels of bacterial contamination based on its superior (non- metallic) taste and appearance.

Taulo *et al.,* (2008 examined water management practices at all the investigated points. One hundred and eighty (180) water samples were collected from 6 villages and tested for *Escherichia coli, Salmonella*, *E. coli* 0157:H7 and *Campylobacter jejuni* using standard methods. Water contamination practices were observed in two hundred and

eighty seven households. *E. coli, Salmonella, E. coli* 0157:H7 and *C. jejuni* were isolated in 54, 24, 6.7 and 2.2% of the samples, respectively. *S*ampling points revealed a significant difference (p = 0.001) in *E. coli* concentration. *Salmonella* concentration between sampling points was not significant ($p > 0.05$). *E. coli* concentration was significantly (p = 0.042) higher than that of *Salmonella* spp. The microbiological quality of water was found to be poor as a result of both poor water management practices and environmental sanitation. There were no significant differences ($p > 0.05$) in water management practices among the villages.

Ishii *et al.,* (2008) studied that *E. coli* can become "naturalized" to soil, sand, sediments, and algae in tropical, subtropical, and temperate environments. This phenomenon raises issues concerning the continued use of this bacterium as an indicator of fecal contamination. In this review, we discuss the relationship between *E. coli* and fecal pollution and the use of this bacterium as an indicator of fecal contamination in freshwater systems. We also discuss recent studies showing that *E. coli* can become an active member of natural microbial communities in the environment, and how this bacterium is being used for microbial source tracking. We also discuss the impact of environmentally-"naturalized" *E. coli* populations on water quality.

Prasai *et al.,* (2007) evaluated the quality of drinking water of the valley of Kathmandu .A total of 132 drinking water samples were randomly collected from 49 tube wells, 57 wells, 17 taps and 9 stone spouts in different places of Kathmandu valley. The samples were analyzed for microbiological parameters. Total plate and coliform count revealed that 82.6% and 92.4% of drinking water samples found to cross the WHO guideline value for drinking water. During the study, 238 isolates of enteric bacteria were identified, of which 26.4% were *Escherichia coli,* 25.6% were *Enterobacter* spp, 23% were *Citrobacter* spp, 6.3% were *Pseudomonas aeruginosa,* 5.4% were *Klebsiella* spp, 4.0% were *Shigella* spp, 3.0% were *Salmonella typhi*, 3.0% were *Proteus vulgaris*, 3.0% were *Serratia* spp and 1.0% were *Vibrio cholera*.

September *et al.,* (2007) collected ninety-five biofilm samples from various parts of South Africa were tested for the presence of *Escherichia coli, Aeromonas, Pseudomonas, Salmonella, Shigella* and *Vibrio* spp. Members of these genera were quantified by the three-tube most probable number (MPN) approach using enrichment broths and plating on selective agars. The heterotrophic culturable counts were determined for both the

planktonic and biofilm phases of the samples. Biofilm density varied between 10 and 1.9×10^9 colony forming unit's cm^{-2}. The 16S rRNA identity of the putative pathogenic isolates revealed that high numbers of *Aeromonas, Pseudomonas, Klebsiella* and *Enterobacter* were present, but no putative *Salmonella* and *Shigella* could be confirmed. None of the *Pseudomonas* isolates belonged to the pathogenic *Pseudomonas aeruginosa* or *Pseudomonas mendocina* while the *Aeromonas* isolates showed relatedness to known pathogenic members of this group.

Chan *et al.,* (2007) determined the quality of filtered drinking water by looking into the microbiological aspect and several physicochemical analyses such as turbidity, pH and total suspended solid (TSS). The microbiological analyses were performed to trace the presence of indicator organisms and pathogens such as *Escherichia coli, Streptococcus faecalis* and *Pseudomonas aeruginosa*. All of the water did not comply with the regulations of Food Act as consisted of more than 103-104 cfu/mL for total plate count. However, the total coliforms and *E. coli* were detected lower than 4 cfu/mL and not exceeding the maximum limit of Food Act. While the presence of *S. faecalis* and *P. aeruginosa* were negative in all samples.

Mario *et al.,* (2005) isolated environmental mycobacteria (EM) such as *M. avium, M. kansasii*, and *M. xenopi* have frequently been isolated from drinking water and hospital water distribution systems. Although the presence of EM in tap water has been linked to nosocomial infections and pseudo-infections, it remains unclear if these EM provide a health risk for immunocompromised people, in particular AIDS patients. In this regard, control strategies based on maintenance of an effective disinfectant residual and low concentration of nutrients have been proposed to keep EM numbers to a minimum in water distribution systems.

Schets *et al.,* (2005) determined the presence of *E. coli* O157 in drinking water by using a specific enrichment method. Eleven percent of the samples contained faecal indicators whereas *E. coli* O157:H7 was isolated from 2.7% of the samples that otherwise met the drinking water standards. The *E. coli* O157 positive water supplies were located on camp-sites in agricultural areas with large grazer densities.

Asano *et al.,* (2004) recharged groundwater with reclaimed municipal wastewater presents a wide spectrum of technical and health alleges that must be carefully evaluated prior to undertaking a project. This review will provide a discussion of groundwater

recharge and its management with special reference to health and regulatory pects of groundwater recharge with reclaimed municipal wastewater. At present, some uncertainties with respect to health risk inside rations have limited expanding use of reclaimed municipal waste water for ground water recharge, especially when a large portion of the groundwater contains reclaimed stem water that may affect the domestic water supply. The proposed State of California criteria for groundwater recharge are discussed as an illustration of a cautious approach.

Jagals *et al.,* (2003) investigated the occurrence of biofilm in PVC storage containers as one possible reason for this deterioration, using heterotrophic bacteria and total coliform counts as well as turbidity as indicators. A second objective was to determine whether biofilm in water-storage containers could contribute to hazardous microbiological contamination indicated by *Escherichia coli* and *Clostridium perfringens*. Results indicated that increased microbiological contamination is associated with biofilm. The biofilm harbours heterotrophic bacteria, total coliforms and *C. perfringens, E. coli* could not be associated directly with the levels of biofilm in containers but rather appears to be introduced intermittently from the ambient domestic environment. When dislodged with the biofilm, these bacteria contributed substantially to the deterioration of the microbiological quality of supplied water stored in plastic containers.

Lejeune *et al.,* (2001) evaluated 473 cattle water troughs located at 99 different cattle operations. The mean $\log_{10}$-transformed coliform and *Escherichia coli* concentrations per milliliter of trough water were 1.76 ± 1.25 (SD) and 0.98 ± 1.06 (SD), respectively. The degree of *E. coli* contamination was positively associated with the proximity of the water through to the feedbunk, protection of the trough from direct sunlight, lower concentrations of protozoa in the water, and warmer weather. *Salmonella* sp. were isolated from 2/235 (0.8%) troughs and shigatoxigenic-*E. coli* O157 was recovered from 6/473 (1.3%) troughs. Four experimental microcosms simulating cattle water troughs were used to further evaluate the effects of protozoal populations on the survival of *E. coli* O157 in cattle water troughs. *Escherichia coli* O157 of bovine fecal origin proliferated in all microcosms. Reduction of protozoal populations by treatment with cycloheximide was associated with increased persistence of *E. coli* O157 concentrations in the microcosms.

Close *et al.,* (2008) assessed groundwater samples (135) were collected, mostly during the irrigation season, with *E. coli* being detected in 75% of samples. *Campylobacter* was identified in 16 samples (12%). A risk assessment of drinking water with these levels of *Campylobacter* was undertaken. A probability distribution was fitted to the observed *Campylobacter* data and the @RISK modeling software was used, assuming a dose response relationship for *Campylobacter* and consumption of 1 L/day of water. The probability of infection on any given day in the study area was estimated at 0.50% to 0.76%, giving an estimated probability of infection during the irrigation season of 60% to 75%. An epidemiological assessment of the Canterbury region comparing areas encompassing dairy within major irrigation schemes (∼55% border-strip irrigation) to two control groups was undertaken. Control group 1 (CG1) encompasses areas of dairying without major irrigation schemes, and a second larger control group (CG2) comprises the rest of the Canterbury region. Comparisons of the subject group to control groups indicated that there was a statistically significant increase in age-standardised rates of campylobacteriosis (CG1 Relative Risk (RR) = 1.51 (95% CI = 1.31-1.75); CG2 RR = 1.51 (1.33–1.72)); cryptosporidiosis (CG1 RR = 2.08 (1.55–2.79); CG2 RR = 5.33 (4.12–6.90)); and salmonellosis (CG2 RR = 2.05 (1.55–2.71)).

Van *et al.,* (2013) described the complex dynamics of microorganisms in water distribution systems. Water quality is diminished primarily as a result of faecal contamination and rarely as a result of putrefaction in water distribution systems. The design of such systems (with/ without anti-backflow valves and pressure) and the materials used (polyethylene enhances biofilm; stainless steel does not) affect the quality of water they provide. The best option is an open, funnel-shaped galvanized drinking trough, possibly with a pressure system, air inlet, and anti-backflow valves. A poor microbiological quality of drinking water may adversely affect feed intake, and herd health and productivity. In turn, public health may be affected because cattle can become a reservoir of microorganisms hazardous to humans, such as some strains of E. coli, Yersinia enterocolitica, and Campylobacter jejuni.

Gharibi *et al.,* (2012) observed that the water quality of Karun River lies in the low to medium range (annual mean index values of 38–55). In addition, the values from the fuzzy DCWQI were generally lower than the values from the NSF WQI, mainly because the DCWQI included heavy metals in its index, while the NSF WQI did not. Results of the present study suggest that DCWQI can be considered as a comprehensive tool for

assessing the quality of water for dairy cattle drinking purposes and can be reliably used for that objective.

Houlbrooke *et al.,* (2004) measured concentration of N and P in drainage water was higher than the ecological limits considered likely to stimulate unwanted aquatic weed growth. Gaps in the current research have been identified with respect to the application of FDE to artificially drained soils, and the lack of research that has taken place with long term application of FDE to land and at appropriate farm scale with realistic rates of application. Whilst the land treatment of FDE represents a huge improvement on the loss of nutrients discharged to fresh water compared with standard two- pond systems, there is room for improvement in the management of FDE land treatment systems

Esterhuizen *et al.,* (2012) measured drinking water quality, including physical, chemical and microbiological properties, on 75 dairy farms in the greater Mangaung region of the Free State, South Africa. Borehole drinking water samples were collected during autumn and spring of 2009 and the physical, chemical and microbiological parameters analysed and compared to the required standards prescribed by the South Africa National Standards (SANS) 241 of 2006. Most farms were compliant; however for combined nitrate and nitrite N, 37 of the farms exceeded the prescribed limit. Similarly, for total coliforms, 45, and for E. coli, 22 of the farms exceeded the acceptable limits. Nine of the farm boreholes were contaminated by N and E. coli. On two of the farms four of the chemical parameters exceeded the prescribed limits, including those for N; both farms were, however, compliant for E. coli**.**

Musa *et al., (*2014) evaluated the quality of the drinking water in dairy farms based on bacteriological examinations and viable counts. A total of 39 water samples were obtained from dairy farms (13 in Khartoum, 13 in Omdurman and 13 in Khartoum North). All samples were cultured on Blood Agar and Mac Conkey for bacterial isolation and on nutrient agar for viable counts. The main result revealed that 39 bacterial isolates were detected in drinking water of the dairy farms in Khartoum state. *Micrococcus* spp., *Pseudomonas* spp., and *Bacillus* spp. were dominant in Khartoum, giving a percentage of 7.69 (n=3) for each. *Staphylococcus* spp. and *Corynobacterium* spp. were also observed in the samples of dairy farms in Khartoum (5.12%) (n=2) for each. The bacteria isolated from dairy farms in Omdurman were *Micrococcus* spp. and *Aeromonas* spp. (5.12%) (n=2) for each. In the same site, *Staphylococcus* spp., *Actenobacillus* spp., *Moraxella*

spp. an *Flavibacterium* spp. were also detected with percentage of 2.56% and frequency of one for each. The most frequent isolate in Khartoum North was *Micrococcus* spp. (17.95%) (n=7) followed by *Aeromonas* spp. (10.26%) (n=4) and *Staphylococcs* spp. (5.12%) (n=2). Regarding bacterial counts, the results have shown high level of contamination of drinking water for all dairy farms in Khartoum State. The results were interoperated, depending on international critical level (cut-off point) (100 CFU ml-1). For instance, high mean of bacterial counts 6.44×10^8 was observed in dairy farm in Omdurman, followed by Khartoum and Khartoum North, with mean bacterial counts of 4.93×10^8 and 3.81×10^8, respectively.

Davies Colley *et al.,* (2014) observed water quality impact of a herd of 246 dairy cows crossing a stream ford was documented. Two cow crossings produced plumes of turbid water associated with very high concentrations of faecal indicator bacteria (*Escherichia coli*) and high suspended solids (SS) and total nitrogen (TN). On the first crossing, towards the milking shed, the cows were tightly-bunched and produced a sharp spike of contamination (*E. coli* peaking at 50 000 cfu/100 ml). After milking, the cows wandered back across the stream as individuals or small groups, and contaminants were less elevated, albeit for a longer period. Light attenuation, measured continuously by beam transmissometer, correlated closely with *E. coli*, SS, and TN, permitting the total yield of these contaminants to be estimated. Contaminant yields for the two crossings were very similar, suggesting that time taken and whether or not cows are herded may not greatly influence water quality impact.

Folorunso *et al.,* (2014) Coumted bacterial on Day 1, for layer chickens on cage system, no significant differences ($p>0.05$) among the farms and between the farms tier interactions. On Day 3, no significant difference ($p>0.05$) among the parameters. On Day 5, there was significant difference ($p<0.05$) among the farms and on Day 7, there was high significant difference ($p<0.01$) among the farms. On Days 5 and 7, there were no significant differences ($p>0.05$) among the tiers nor between the interactions of the farms and tiers. The bacterial count in water troughs of layer chickens in deep litter system, on Day 1, had no significant differences ($p>0.05$) between the farms, water troughs and their interactions. On Day 3, no significant difference ($p>0.05$) among the parameters. On Days 5 and 7, there were significant difference ($p<0.05$) and a high significant difference ($p<0.01$) between the farms respectively. On Days 5 and 7, no significant differences between the water troughs and between the interaction of the farms and the

water troughs. Farm A isolates contained *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermis*, *Klebsiella* sp., *Salmonella* sp., *Bacillus subtilis*, *Lactobacillus salivarius* and *Corynebacterium* sp. Farm B had *Staphylococcus aureus*, *S. epidermis*, *Bacillus subtilis*, *Corynebacterium* sp., *Escherichia coli*, *Streptococcus faecalis* and *Klebsiella* sp.

Sparks *et al.,* (2009) Studied the role of water in Campylobacter infection of chickens have identified the importance of factors such as biofilm in protecting the organisms and, potentially, the viable but non-culturable form (VNC) of Campylobacter. While difficulties in identifying the VNC form in field outbreaks may have led to an underestimate of the importance of water as a risk factor there are contradictory views regarding the ability of the VNC form to cause infection under field conditions. Producers may treat drinking water with a range of products to reduce the number of microbial contaminants, Campylobacter included, that reach the growing bird in the drinking water. Examples of products used by producers include chlorine, chlorine dioxide, organic acids, peracetic acid and hydrogen peroxide. The efficacy of these products differs depending on the environment in which they are used, pH for example having a significant effect on the efficacy of chlorine. If the full benefits of drinking water treatment are to be realised then further evidence is required, in terms of the effects on the normal production parameters such as weight gain, feed conversion efficiency, flock uniformity as well as on the prevalence of infection such as that caused by Campylobacter.

2.2 To characterization of identified isolates by using PCR

Wang *et al.,* (2018) collected four water samples with different water ages, including finished water (FW, 0 d) and tap water (TW) [TW1 (1 d), TW2(2 d) and TW3(3 d)], along with the mains of a practical DWDS, and the bacterial community was investigated by high-throughput sequencing technique. Results indicated that the residual chlorine declined with the increase of water age, accompanied by the increase of dissolved organic matter, total bacteria counts and bacterial diversity (Shannon). For bacterial community composition, although *Proteobacteria phylum* (84.12%-97.6%) and Alphaproteobacteria class (67.42%-93.09%) kept dominate, an evident regular was observed at the order level. In detail, the relative abundance of most of other residual orders increased with different degrees from the start to the end of the DWDS, while a

downward trend was uniquely observed in terms of Rhizobiales, who was inferred to be chlorine-resistant and be helpful for inhibiting pipes corrosion. This paper revealed bacterial community variations along the mains of the DWDS and the result was helpful for understanding bacterial ecology in the DWDS.

Lyimo *et al.,* (2016) identified the replicon types of plasmids, conjugation efficiencies, and the complement of antibiotic resistance genes for a panel of multidrug resistant E. coli isolates from surface waters in northern Tanzania. Standard membrane filtration was used to isolate and uidA PCR was used to confirm the identity of strains as E. coli. Antibiotic susceptibility was determined by breakpoint assay and plasmid conjugation was determined by filter-mating experiments. PCR and sequencing were used to identify resistance genes and PCR-based replicon typing was used to determine plasmid types. Filter mating experiments indicated conjugation efficiencies ranged from 10^{-1} to 10^{-7}. Over 80% of the donor cells successfully passed their resistance traits and eleven different replicon types were detected (IncI1, FIC, P, FIIA, A/C, FIB, FIA, H12, K/B B/O, and N). Inc F plasmids were most commonly detected (49% of isolates), followed by types IncI1 and Inc A/C.

Fish *et al.,* (2015) produced a matrix of extracellular polymeric substances (EPS) by the attached community and provides structure and stability for the biofilm. If the EPS adhesive strength deteriorates or is overcome by external shear forces, biofilm is mobilized into the water potentially leading to degradation of water quality. However, little is known about the EPS within DWDS biofilms or how this is influenced by community composition or environmental parameters, because of the complications in obtaining biofilm samples and the difficulties in analyzing EPS. This research applies an EPS analysis method based upon fluorescent confocal laser scanning microscopy (CLSM) in combination with digital image analysis (DIA), to concurrently characterize cells and EPS (carbohydrates and proteins) within drinking water biofilms from a full-scale DWDS experimental pipe loop facility with representative hydraulic conditions. Application of the EPS analysis method, alongside DNA fingerprinting of bacterial, archaeal communities, was demonstrated for biofilms sampled from different positions around the pipeline, after 28 days growth within the DWDS experimental facility. The volume of EPS was 4.9 times greater than that of the cells within biofilms, with carbohydrates present as the dominant component.

Banihashemi *et al.,* (2015) measured bacterial enteric pathogens in a river in southern Ontario, Canada that is used as a source of drinking water by a cell viability assay. Pathogen concentrations were measured using both propidium monoazide (PMA)-quantitative polymerase chain reaction (PCR) and quantitative PCR (qPCR) without PMA pretreatment to compare viable and total (live and dead) cells. The pathogens evaluated were *Salmonella enterica*, thermophilic *Campylobacter*, and *Escherichia coli*O157:H7, and the suspected enteric pathogen *Arcobacter butzleri* was also investigated. Results showed that for all strains dead cells were detected in few river water samples, and the difference between total and viable cell concentrations for each pathogen group was always less than 0.5 log. *A. butzleri* was detected at concentrations 2–3 log higher than the other pathogens. *S. enterica*, *Campylobacter*, and *E. coli* O157:H7 were detected at low concentrations at one sample location and at higher concentrations at a second sampling location.

Silva *et al.,* (2015) monitored the microbiological safety of water are based on culturing the microorganisms. However, these methods are not the desirable solution to prevent outbreaks as they provide the results with a considerable delay, lacking on specificity and sensitivity. Moreover, viable but non-cultural microorganisms, which may be present as a result of environmental stress or water treatment processes, are not detected by culture-based methods and, thus, may result in false-negative assessments of E. coli in water samples. Molecular methods, particularly polymerase chain reaction based methods, have been studied as an alternative technology to overcome the current limitations, as they offer the possibility to reduce the assay time, to improve the detection sensitivity and specificity, and to identify multiple targets and pathogens, including new or emerging strains.

Rani *et al.*, (2014) molecular-beacon based qPCR assay targeting *staG* gene was designed for specific detection and quantification of *S.* Typhi and validated against water and sediment samples collected from the river Ganga, Yamuna and their confluence on two days during Mahakumbha mela 2012–2013 (a) 18 December, 2012: before six major religious holy dips (b) 10 February, 2013: after the holy dip was taken by over 3,00,00,000 devotees led by ascetics of Hindu sects at Sangam on 'Mauni Amavasya' (the most auspicious day of ritualistic mass bathing). The assay could detect linearly lowest 1 genomic equivalent per qPCR and is highly sensitive and selective for *S. typhi* detection in presence of non-specific DNA from other bacterial strains including *S.*

paratyphi A and *S. typhimurium*. It has been observed that water and sediment samples exhibit *S. typhi*. The mass holy dip by devotees significantly affected the water and sediment quality by enhancing the number of *S. typhi* in the study area. The qPCR developed in the study might be helpful in planning the intervention and prevention strategies for control of enteric fever outbreaks in endemic regions.

Holinger *et al.,* (2014) sampled tap water from seventeen different cities between the headwaters of the Arkansas River and the mouth of the Mississippi River and determined the bacterial compositions by pyrosequencing small subunit rRNA genes. Nearly 98% of sequences observed among all systems fell into only 5 phyla: Proteobacteria (35%), Cyanobacteria (29%, including chloroplasts), Actinobacteria (24%, of which 85% were *Mycobacterium* spp.), Firmicutes (6%), and Bacteroidetes (3.4%). The genus *Mycobacterium* was the most abundant taxon in the dataset, detected in 56 of 63 samples (16 of 17 cities). Abundant taxa (excepting Cyanobacteria and chloroplasts) were generally similar from system to system, however, regardless of source water type or local land use. The observed similarity among the abundant taxa between systems may be a consequence of the selective influence of chlorine-based disinfection and the common local environments of DWDS and premise plumbing pipes.

Mulamattathil *et al.,* (2014) analysed water samples from five different sites for the presence of faecal indicator bacteria as well as Aeromonas and Pseudomonas species. Faecal and total coliforms were detected in summer in the treated water samples from the Modimola dam and in the mixed water samples, with Pseudomonas spp. being the most prevalent organism. The most prevalent multiple antibiotic resistance phenotype observed was KF-AP-C-E-OT-K-TM-A. All organisms tested were resistant to erythromycin, trimethoprim, and amoxicillin. All isolates were susceptible to ciprofloxacin and faecal coliforms and Pseudomonas spp. to neomycin and streptomycin. Cluster analysis based on inhibition zone diameter data suggests that the isolates had similar chemical exposure histories.

Lu *et al.,* (2014) developed quantitative polymerase chain reaction assay (115 bp amplicon) specific to *Escherichia coli* K12 with an ABI^{TM} internal control based on sequence data encoding the *rfb* gene cluster. Assay specificity was evaluated using three *E. coli* K12 strains (ATCC W3110, MG1655 & DH1), 24 non-K12 *E. coli* and 23 bacterial genera. The biofilm detection limit was 10^3 colony-forming units (CFU) *E. coli*

K12 mL^{-1}, but required a modified protocol, which included a bio-blocker *Pseudomonas aeruginosa* with ethylenediaminetetraacetic acid buffered to pH 5 prior to cell lysis/DNA extraction. The novel protocol yielded the same sensitivity for drinking water biofilms associated with Fe_3O_4 (magnetite)-coated SiO_2 (quartz) grains and biofilm-surface iron corrosion products from a drinking water distribution system. The novel DNA extraction protocol and specific *E. coli* K12 assay are sensitive and robust enough for detection and quantification within iron drinking water pipe biofilms, and are particularly well suited for studying enteric bacterial interactions within biofilms.

Popovici *et al.,* (2014) characterized unique bacterial species and are a reflection of the large diversity of surface structures, proteins, and appendages of microorganisms. CSH and EPM of bacterial cells contribute substantially to the effectiveness of drinking water treatment to remove them, and therefore an investigation of these properties will be useful in predicting their removal through drinking water treatment processes and transport through drinking water distribution systems. EPM and CSH measurements of six microbiological pathogen or surrogate species suspended in phosphate-buffered water are reported in this work. Two strains of *Vibrio cholerae* were hydrophobic, while three strains of *Escherichia coli* were hydrophilic. *Bacillus cereus* was categorized as moderately hydrophobic. The strains of *E. coli* had the highest (most negative) EPM. Based on the measurements, *E. coli* species is predicted to be most difficult to remove from water while *V. cholerae* will be the easiest to remove.

Maheux *et al.,* (2014) demonstrated the ability of a bacterial concentration and recovery procedure combined with three different PCR assays targeting the lacZ, wecG, and 16S rRNA genes, respectively, to detect the presence of total coliforms in 100-ml samples of potable water (presence/absence test). PCR assays were first compared to the culture-based Colilert and MI agar methods to determine their ability to detect 147 coliform strains representing 76 species of Enterobacteriaceae encountered in fecal and environmental settings. Results showed that 86 (58.5%) and 109 (74.1%) strains yielded a positive signal with Colilert and MI agar methods, respectively, whereas the lacZ, wecG, and 16S rRNA PCR assays detected 133 (90.5%), 111 (75.5%), and 146 (99.3%) of the 147 total coliform strains tested. These assays were then assessed by testing 122 well water samples collected in the Québec City region of Canada. Results showed that 97 (79.5%) of the samples tested by culture-based methods and 95 (77.9%), 82 (67.2%), and 98 (80.3%) of samples tested using PCR-based methods contained total coliforms,

respectively. Consequently, despite the high genetic variability of the total coliform group, this study demonstrated that it is possible to use molecular assays to detect total coliforms in potable water: the 16S rRNA molecular assay was shown to be as efficient as recommended culturebased methods.

Jebelli *et al.,* (2012) designed primer related to genomic segment of Lac Z it is duplicated using PCR technique in order to isolate bacteria from samples of drinking water. This genomic segment encodes β-galactosidase enzyme in Colifrom bacteria. Then results are compared with multiple-tube fermentation technique by MPN method. Sampling was performed in 18 wells of drinking water in Qom's villages and samples were tested using PCR and MPN techniques. Of the total samples tested, 3 samples responded positively to MPN technique and 8 samples responded positively to PCR technique. Findings suggest higher accuracy in PCR compared to MPN.

McMahana *et al.,* (2012) identified the types and numbers of microbial community members present in natural water samples, including fecal indicators and pathogens as well as other bacteria. Representative water sources tested in this study included cistern rainwater, a protected lake, and wells in agricultural and forest settings. Samples from quantitative H2S tests of water were further cultured for fecal bacteria by spread plating onto the selective media for detection and isolation of *Aeromonas* spp.*, E. coli, Clostridium* spp., H2S-producers, and species of *Salmonella* and *Shigella.* Isolates were then tested for H2S production, and identified to the genus and species level using biochemical methods. Terminal Restriction Fragment Length Polymorphisms (TRFLP) was the molecular method employed to quantitatively characterize microbial community diversity. Overall, it was shown that water samples testing positive for H2S bacteria also had bacteria of likely fecal origin and waters containing fecal pathogens also were positive for H2S bacteria. Of the microorganisms isolated from natural water, greater than 70 percent were identified using TRFLP analysis to reveal a relatively stable group of organisms whose community composition differed with water source and over time.

Ozgumus *et al.,* (2007) isolated a hundred and seventeen antibiotic-resistant Escherichia coli strains from public tap and spring waters which were polluted by fecal coliforms. There were no significant differences between two water sources as to the coliform pollution level ($p > 0.05$). All E. coli isolates were detected to be resistant to one or more antibiotics tested. Nearly 42% of the isolates showed multiresistant phenotype. Three

(2.5%) of these isolates contained class 1 integron. Sequencing analysis of variable regions of the class 1 integrons showed two gene cassette arrays, dfr1-aadA1 and dhfrA17-aadA5. Genotyping by BOX-polymerase chain reaction (BOX-PCR) showed that some of the strains were epidemiologically related. This is the first report on the prevalence and characterization of class 1 integron-containing *E. coli* isolates of environmental origin in Turkey.

Bhatta *et al.,* (2007) detected Occurrence of *Salmonella* in 42 out of 300 water samples by enrichment culture technique in selenite F broth followed by plating on Salmonella Shigella agar. A total of 54 isolates identified to genus level by standard tests were subsequently confirmed by serotyping, phage typing and PCR detection of virulence genes. The predominant serotype was *Salmonella typhimurium*, followed by *Salmonella typhimurium*, *Salmonella paratyphi* A and *Salmonella Enteritidis*. Most of the *Salmonella typhimurium* isolates were E1 phage type followed by UVS4, A and UVS1. All isolates of *Salmonella paratyphi* A and *Salmonella enteritidis* were an untypable (UT) phage type.

Domingo *et al.,*(2003) monitored the impact of chlorination and chloramination treatments on heterotrophic bacteria (HB) and ammonia-oxidizing bacteria (AOB) inhabiting a water distribution system simulator. HB densities decreased while AOB densities increased when chloramine was added. AOB densities decreased below detection limits after the disinfection treatment was switched back to chlorination. The presence of AOB was confirmed using a group-specific 16S rDNA-PCR method. 16S rDNA sequence analysis showed that most bacterial isolates from feed water, discharge water, and biofilm samples were α-Proteobacteria or β-Proteobacteria. The latter bacterial groups were also numerically dominant among the sequences recovered from water and biofilm 16S rDNA clone libraries. The relative frequency of each culturable bacterial group was different for each sample examined. Denaturing gradient gel electrophoresis analysis of total community 16S rDNA genes showed notable differences between the microbial community structure of biofilm samples and feed water. The results of this study suggest that disinfection treatments could influence the type of bacterial community inhabiting water distribution systems.

Toze *et al.,* (1999) determine indicator bacteria which are commonly used to detect the relative risk of faecal contamination and the possible presence of pathogens in water and

wastewaters. Indicator organisms, however, have several disadvantages that make them less than ideal for indicating the possible presence of microbial pathogens. Consequently methods to directly detect microbial pathogens in water and wastewaters are being investigated. Polymerase chain reaction (PCR) is one of the main alternative detection methods being trialed. PCR has been shown to be a rapid, highly sensitive and accurate method. It has already been used experimentally to detect pathogenic viruses, bacteria and protozoa in water and wastewaters. PCR, however, has a number of limitations. One such limitation is the generation of false positives through the detection of naked nucleic acids, non-viable microorganism, or through contamination in the laboratory.

Rodriguez *et al.,* (2012) Collected supply water and wastewater from 20 dairy farms from Antioquia, Colombia was monitored for 10 months to determine the presence of pathogenic microorganisms. Both *Cryptosporidium* and *Fasciola* were determined by the Polymerase Chain Reaction (PCR) technique in real time. The results showed that the supply water used for drinking and activities involving the herd, has high populations of *Fasciola hepatica* and *Cryptosporidium parvum*, with percentages of about 53.7% and 64.75% respectively. Additionally high populations of *Pseudomonas aeruginosa*, *Shigella*, *Salmonella*, total coliforms and Escherichia coli were found in both types of water, with values around 9.4×10^7, 2.1×10^7, 1.8×10^7, 1.9×10^{10} and 1.5×10^{10} UFC/100 ml respectively for the wastewater and 3.1×10^4, 1.9×10^4, 7.3×10^3, 1.2×10^5 and 6.2×10^3 UFC/100 ml for the supply water.

CHAPTER 3

MATERIALS AND METHODS

The research work was conducted during the period from July 2018 to July 2019 at the bacteriological laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), dinajpur. The details outline of the materials and methods are given below:

3.1 Materials used

3.1.1 Study area and period

The water samples were collected from the different tubewell(human), manger (animal farm) and waterer (poultry farm) at different upazilla in Dinajpur district and brought to the laboratory under aseptic condition for laboratory analysis. The research work was conducted during the period from July 2018 to july 2019.

Fig. 3.1: Study area map

3.1.2 Research design

Completely Randomized Design (CRD) and descriptive cross-sectional survey were used to isolate and identify the bacteria from water samples. The design was chosen because

the study was concerned with identification of pathogen from water sample by using cultural, biochemical and staining techniques.

3.1.3 Sample collected from different tubewell of human use, manger of dairy farm and waterer of poultry farm

A total of 90 samples were collected from different tubewell of human use, manger of dairy farm and waterer of poultry farm at different upazilla in Dinajpur district. The samples were collected under aseptic condition with the help of pre sterilized screw cap test tube and immediately transferred into the microbiology laboratory, HSTU. From each sampling point 250 ml water samples were taken for analyses. The bacteriological tests were undertaken within 6 hours after collection to avoid the growth or death of microorganisms in the sample.

3.1.4 Materials used for sample collection

Fig. 3.2: Ice box

3.1.4.1 Glassware and appliances

The glassware's and appliances were used during the whole period of the experiment are as follows: scalpel, forceps, scissors, tray, petri dishes, test tubes, conical flask, pipette, micro pipette, slides, test tube racks, water bath, bacteriological incubator, refrigerator, sterilizing instruments, hot air oven, centrifuge tubes and machine, ice boxes, electronic balance, syringe and needle, compound microscope, spirit lamps, match lighter, bacteriological loop, inoculum loop, autoclave machine, filter paper.

3.1.4.2 Chemicals and reagents

The chemicals and reagents used for the study were Gram's stains (Gram's iodine, safranin, acetone alcohol, and immersion oil), Methyl Red-Voges proskaur (MR-VP)

solution, Kovac's indole reagent, alcohol, glycerin and other common laboratory reagents and chemicals.

3.1.5 Media for culture

3.1.5.1 Liquid Media

Nutrient broth

The medium was prepared by adding 13 g of nutrient broth powder to one liter of distilled water and well mixed. The pH was adjusted to 7.4. The mixture was distributed in 5 m1 volumes into clean bottles, and then sterilized by autoclaving at 121 °C (15 lb/inch^2) for 15 minutes.

Peptone water

This medium was prepared by dissolving 10 g of peptone water and 5g sodium chloride in 1litre of distilled water. The mixture was distributed in 5 ml volumes into clean bottles, and sterilized by autoclaving at 121°C (15lb/inch^2) for 15 minutes.

3.1.5.2 Solid media

Nutrient agar

Twenty-eight grams of nutrient agar powder (Hi-media, India) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37°C for overnight to check their sterility and used for culture characterization (Carter, 1979).

Plate Count Agar (PCA)

Add 17.5g to 1 liter of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes. After autoclaving, the medium was poured into each sterile petri dish and allowed to solidify. After solidification of the medium in the petri dishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C refrigerator for future use (Cater 1979).

MacConkey agar

51.50 grams of dehydrated Bacto-MacConkey agar (Difco) was suspended in 1000 ml of cold distilled water taken in a conical flask and was heated up to boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured sterile glass petridishes. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

Eosin methylene blue agar

Eosin methylene blue (EMB) agar medium was used to observe the growth of *Escherichia coli* (Cheesebrough, 1985). 36 grams of EMB agar base (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in to sterile glass. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used or stored at 4°C in refrigerator for future use (Carter, 1979).

Salmonella Shigella Agar

Salmonella Shigella (SS) agar is the selective medium for the isolation of *Salmonella* and *Shigella*. 63.0 grams SS agar powder was dissolved in 1000 ml of distilled water. It was mixed well until a homogeneous suspension is obtained. It was heated with frequent agitation and boiled for one minute. It did not sterilize by autoclaved. It was cooled to 45℃ and 50°C and distributed in Petri plates and allow the medium to solidify partially uncovered. (HIMEDIA and Leifson *et al.,* 1935).

Mannitol Salt Agar

111 grams Mannitol Salt Agar base powder was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely (necessary calculation was done for required number of plates). The medium was then sterilized by autoclaving at 1.2 kg/cm2 pressure and 121° C for 15 minutes. After autoclaving the medium was put into water bath at 450- 500C to decrease the temperature. Then medium was poured in

10 ml quantities in sterile glass petri dishes (medium sized) and in 15 ml quantities in sterile glass Petri dishes (large sized) to form thick layer there. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the Petri dishes partially removed. The sterility of the medium was checked by incubating at 37°C for overnight. The sterile medium was used for cultural characterization or stored at 4°C in refrigerator for future use. Petri dishes, these were incubated at 37° C for overnight to check their sterility and used for cultural characterization or stored at 4°C in refrigerator for future use (Cater 1979).

Cetrimide Agar

46.7 grams of Cetrimide agar powder (Hi-media, India) was suspended in 1000 ml of distilled water containing 10 ml glycerol in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. The precipitate was resuspended by gentle agitation to avoid bubbles and pour the plates while the medium is hot and allowed to solidify. Alternatively, the medium was cooled to 45-50°C. After solidification of the medium in the petridishes, these were incubated at 37°C for overnight to check their sterility and used for culture characterization (Carter, 1979).

Mueller Hinton Agar

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. 38 grams of Mueller Hinton agar powder was suspended in 1000 ml of distilled water. It was mixed well. It was heated agitating frequently and boiled for about one minute. It was dispensed and sterilized in autoclave at 116 - 121°C (15 lbs. sp) for 15 minutes. It was cooled to 45° or 50°C (Carter, 1979).

Thiosulfate-Citrate-Bile salts-Sucrose (TCBS) agar

Suspend 89.08 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely without using autoclave. Then Cool to 45-50°C. Mix well and pour into sterile Petri plates. (Kobayashi 1963).

MIU medium

18 grams of MIU agar (Difco) was suspended in 950 ml of cold distilled water taken in a conical flask and heated up to boiling to dissolve the medium completely. Ninety-five ml was dispensed into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15

minutes. Then was Cooled to about 50-55°C and aseptically add 5ml was added of sterile 40% basal medium. After mixing were dispensed into sterile test tubes. Allow to cool in an upright position. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3.1.6 Reagents preparation

Methyl Red-Voges Proskauer broth

A quantity of 3.4 grams of Bacto MR-VP medium was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the test tubes were autoclaved. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and used for biochemical characterization or stored at 4°C in refrigerator for future use (Cheesbrough,1984).

Methyl Red solution

The indicator methyl red (MR) solution was prepared by dissolving 0.1 gm of Bacto methyl red (Difco) in 300 ml of 95% alcohol and diluting this to 500 ml with the addition of 200 ml of distilled water.

Voges-Proskauer solution

Alpha-naphthol solution

Alpha-naphthol solution was prepared by dissolving 5 grams of 1-naphthol in 100 ml of 95% ethyl alcohol.

Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of potassium hydroxide crystals in 100 ml of cold distilled water.

Indole reagent

Kovac's reagent

This solution was prepared by mixing 25 ml of concentrated Hydrochloric acid in 75 ml of amyl alcohol and to this mixture 5 grams of paradimethyl-aminohenzyldehide crystals

were added. This was then kept in a flask equipped with rubber cork for future use (Merchant and Packer, 1967).

Phosphate buffered saline solution

For preparation of Phosphate buffered saline (PBS) solution, 8 gram of sodium chloride (NaCl), 2.89 gram of disodium hydrogen phosphate (Na_2HPO_4, $12H_2O$), 0.2 gram of potassium chloride (KCl) and 0.2 gram of potassium hydrogen phosphate (KH2PO4) were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely. The solution was then sterilized by autoclave at 121 °C maintaining a pressure of 15 pounds per square inch for 15 minutes and stored at refrigerator until use. The pH of the solution was measured by a pH meter and maintained at 7.0-7.2 (Cheesbrough, 1984).

3.2 Methods

3.2.1 Laboratory preparation

All items of glassware including test tubes , pipettes, cylinder, flasks, conical flasks, glass plate, slides, vials and agglutination test tubes soaked in a household dishwashing detergent solution for overnight, contaminated glassware were disinfected in 2% sodium hypochloride solution prior to cleaning .The glassware were then cleaned by brushing ,washed thoroughly and finally sterilized either by dry heat at 160°C for 2 hours or by autoclaving for 15 minutes at 121°C under 15 lbs pressure per square inch. Autoclaved items were dried in a hot air oven at 50°C.Disposable plastic ware (micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50°C for future use.

3.2.2 Experimental Layout

The present study was conducted into fifth phases; in first phase going to the selected area observed tubewel and tap and taking history recorded. In the second phase performed TVC. In third phase isolation and identification of the organisms from the collected sample using cultural, staining and biochemical characteristics was done. In fourth phase drug resistant pattern of the selected isolates were determined by using different antibiotic discs available in the market. Finally transferred sample (pure culture along with broth) to the Invent com. Ltd bonani Dhaka for PCR. The experimental layout of the present study was shown.

Experimental layout

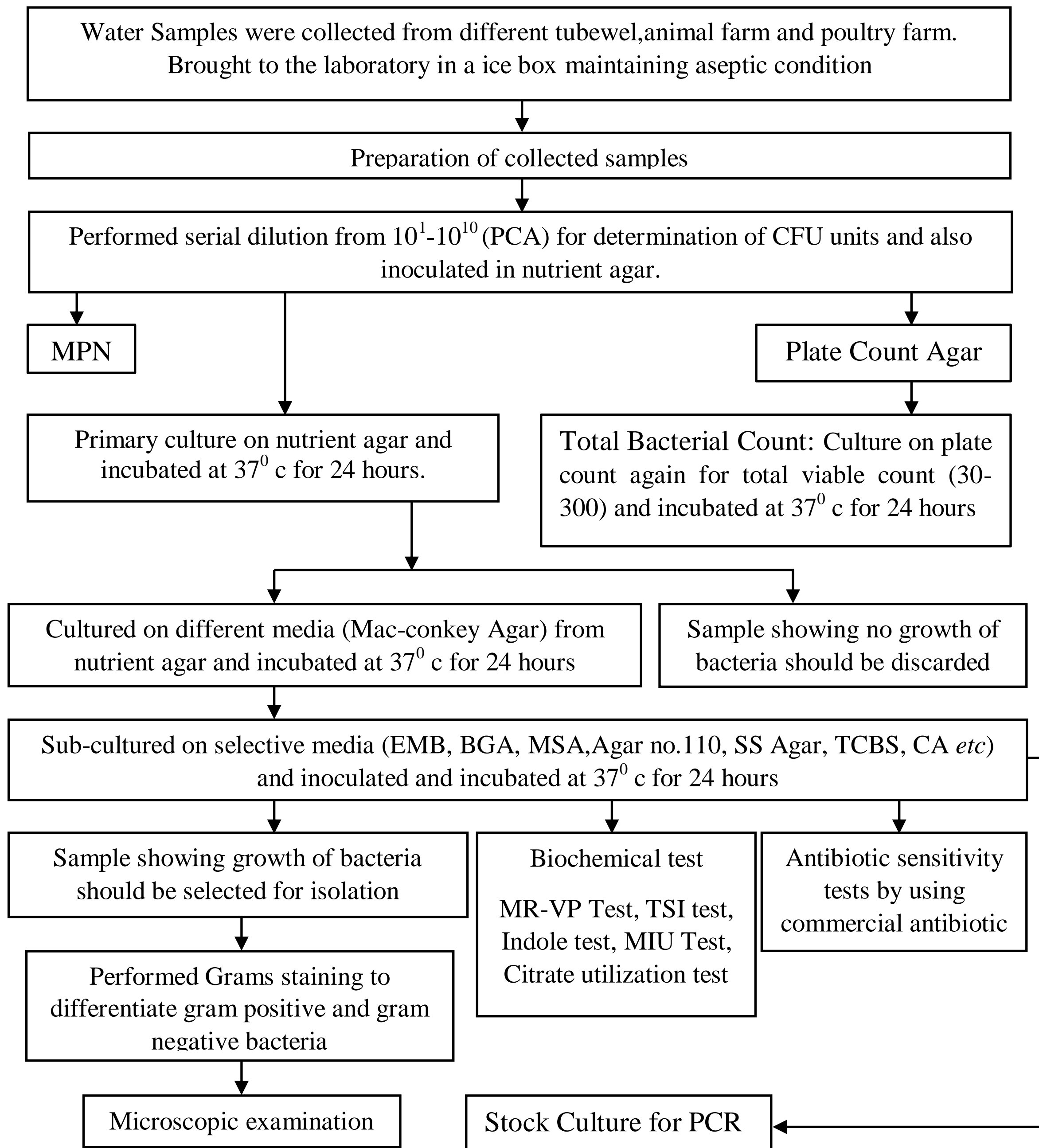


Fig. 3.3: Schematic illustration of the isolation and identification pathogen from water sample

3.2.3 Serial dilution of Sample

10g of each fish sample were weighed aseptically and homogenized in 90ml sterile PBS water. Then, serial dilutions were made by mixing 1.0ml of the suspension in 9.0ml sterile PBS water to obtain 10^{-1} dilution. The dilution was then made to 10^{-2}, and 10^{-6} diluents.

At first for each of the processed samples 10 sterile test tubes were placed on a test tube holder rack containing 9 ml of 2% buffered peptone water.

Fig. 3.4: Serial Dilution

1 ml processed sample was mixed with 9 ml of Phosphate buffer solution in the 1st test tube in order to make 10^{-1} dilution. Then 1ml solution from 1st test tube mixed with 2ndt test tube, then from 2nd test tube to 3rd test tube and finally 5th to 6th test tube and 1ml discard from 7th test tube by the help of pipette and in every step, mixing was done properly.

3.2.4 Enumeration of total viable count (TVC)

To determine the total viable plate count, serial 10-fold dilutions of samples were prepared in physiological saline, and 50 µl (0.05 ml) of aliquot was spread on plate count agar (PCA). Plates were incubated for 24 hours at 37°C before bacteriological counts were done. The number of colonies on each plate having 30–300 colonies was counted by using a digital colony counted. Finally, the bacterial count was reported CFU/mL as follows:

$$\text{CFU} = \frac{\text{Colonies counted} \times \text{Dilution factor}}{\text{Actual volume of sample in plate,(ml)}}$$

Calculation:

Colonies per plate=95

Dilution factor=10^6

Volume of dilution added to plate= 0.5 ml

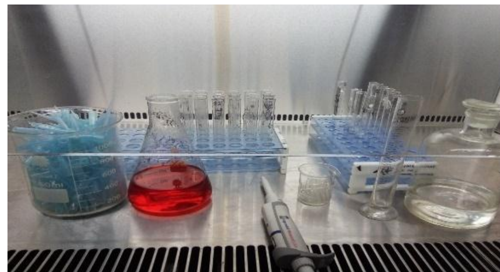
So, $1.9\text{x}10^8$CFU/ml (Colony-forming units).

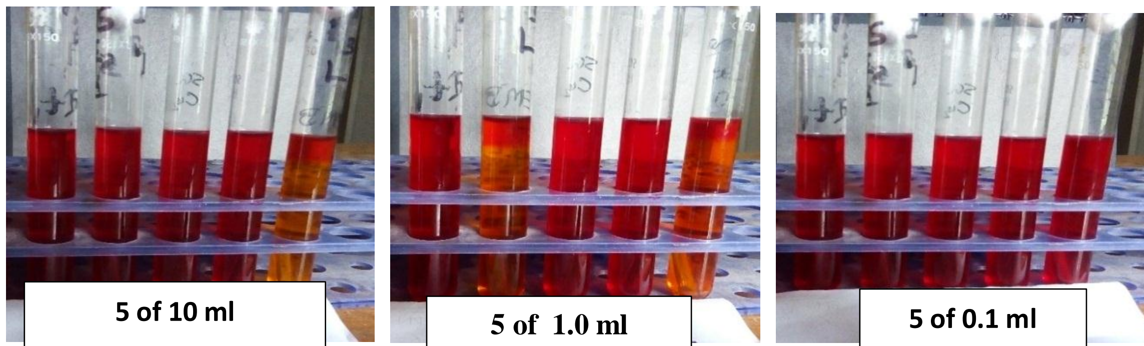
After that, based on colony morphology representative colonies were picked and sub cultured on different selective and differential media such as MacConkey agar, EMB

agar, SS agar, BGA agar, MSA agar CA agar ,TCBS agar, *Staphylococcus* Agar no. 110 etc. After obtaining pure colonies and recording key features and were identified biochemically in a systematic way following standard methods.

3.2.5 Total Coliform Count by MPN Method

Most probable number (MPN) test was used to detect the presence of coliforms in sample water. In presumptive MPN procedure, 15 lactose broth tubeswere 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**.**





Sample conc.	5 of 10ml	5 of 1ml	5 of o.1ml	MPN/100ml
Tube positive	1	2	0	6

Fig. 3.5: Most probable number (MPN)

3.2.6 Culture in ordinary media

Samples were inoculated separately into ordinary media like nutrient agar, nutrient broth and were incubated at 37°C for overnight. The colonies on primary cultures were

repeatedly sub-cultured by streak plate method (Cheesbrough, 1984) until the pure culture with homogenous colonies were obtained.

3.2.7 Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, the mixed culture was inoculated into nutrient agar media by streak plate technique to obtain isolated colonies as per:

Step-1: An inoculum was picked up with a sterile loop and spread on an area of the medium in the petridish.

Step-2: The loop was sterilized by being heated as red hot in a flame.

Step-3: The inoculum was spread over the reminder of the plate by drawing the cooled parallel line.

This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.2.8 Morphological Characteristics of organism by gram's staining method

The gram staining was followed to study the morphological and staining characterization of bacteria and provide information about the presumptive bacterial identification as per recommendation of (Cowan and Steel 1979).

Procedure:

- A loopful sterile distilled water was placed in the center of a clean sterile slide.
- A small colony was picked up with a bacteriological loop and was mixed with distilled water of a slide.
- The colony was made to thin smear on a slide.
- The smears were fixed by air driving.
- 0.5% of crystal violet solution was then applied on the for two minutes
- Then washout with clean water
- Grams iodine was then added to act as mordant for one minute
- Then washed out with clean water
- Acetone alcohol was then added to decolorize for 1-2 seconds.
- washed out with clean water
- Safranin was as counter a stain and allowed for one minute.
- Washed out with water.

➢ Then the slide was blotted with blot paper and was allowed to dry. The slide was examined under microscope with high power objective (100X) using with immersion oil.

Grams staining observation:

Gram Positive: Dark purple.
Gram Negative: Pale to dark red.
Cocci: Round shape.
Bacilli: Rod shape.

3.2.9 Culture into differential media

Mac-Conkey agar

Samples were sub-culture on Mac-conkey agar media and incubated at 37°C for overnight. After that both lactose fermenter bacteria (rose pink color colony) and lactose non-fermenter bacteria (pale color colony) were selected.

3.2.10 Culture on selective media

3.2.10.1 Eosin Methylene Blue (EMB) agar

Samples of positive lactose fermenter were taken and sub-culture on EMB agar media and incubated at 37°C for overnight. Some EMB agar plate showed slightly circular colonies with dark center metallic sheen. Also, in some EMB agar, the growth was indicated by smooth, characteristics mucoid and pink colored colonies which are a consequence of the organism's abundant polysaccharide capsule.

3.2.10.2 Salmonella -Shigella agar

Sample of non-lactose fermenter were taken and sub-culture on SS agar media and incubated at 37°C for overnight, which after inoculation, raised, black centered, smooth round colony was present.

3.2.10.3 Mannitol salt agar (MSA)

Materials from nutrient agar were inoculated into Mannitol salt agar containing plates and incubated at 37°C for overnight, which after inoculation, raised, yellow, smooth colony was present.

3.2.10.4 Agar No. 110

Materials from nutrient agar were inoculated into Agar No.110 containing plates and incubated at 37°C for overnight, which after inoculation, raised, grayish, smooth colony was present.

3.2.10.5 Cetrimide agar

Cetrimide Agar is the selective agent as it inhibits the growth of the accompanying microbial flora. Typical *Pseudomonas* spp colonies are greenish or yellowish green in color (Cheesbrough, 1985).

3.2.10.6 Thiosulfate-citrate-bile salts-sucrose (TCBS) agar

Samples were inoculated int TCBS agar plates after incubation (37^{0}c for overnight) the yellow pigmented colonies indicated positive test for *Vibrio species*.

3.2.11 Identification of isolates

The purified isolates were identified according to criteria described by Barrow and Feltham (1993). This included staining reaction, cell morphology, cultural characteristics and biochemical characteristics.

3.2.11.1 Microscopic examination

Smears were made from each type of colony on primary culture and from purified colonies. Then fixed by heating and stained by Gram staining method as described by Barrow and Feltham (1993). The stained smears were examined microscopically under oil immersion lens for cell morphology, cell arrangement and staining reaction.

3.2.11.2 Biochemical methods for identification of isolated bacteria:

All biochemical tests were performed as described by Barrow and Fellham (1993). They included the following tests:

Catalase test

Indole test

Methyl test

Voges-Proskauer (VP) test

Triple sugar iron (TSI)

Citrate utilization test

Motility indole and ornithine decarboxylate (MIO) test.

3.2.11.2.1 Catalase test

The present of catalase is determined by its ability to break down peroxide into water and oxygen, releasing bubbles of oxygen. This test is used to differentiate those bacteria that produce the enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci* by (Cheesbrough, 1985).

Procedure:

Picked up a colony of the bacteria from a plate and transferred the colony on a glass slide in a drop of water.

Placed a few drops of 3% $H2O2$ (dilute 30% commercial solution (1: 10) over the culture.

Observations: Positive- Immediate strong bubbling; **Negative-** No formation of bubble.

3.2.11.2.2 Indole test

The test organism was cultured in a medium containing tryptophan. The organisms break down tryptophan and indole are released. It was detected by the action of Kovac 's reagent or Ehrlich reagent (formation of red colored compound). This test was important for the identification of Enterobacteria such as *Escherichia coli*, *Pseudomonas vulgaris* etc. by (Cheesbrough, 1985).

Procedure

(a) Tryptophan containing broth was inoculated with bacteria.

(b) The tube was incubated at 37° C for 24 hours.

(c) Added 0.5 ml of the Kovac 's reagent after the bacterial growth.

(d) If indole positive within a 30 second a red color ring appeared at the junction of medium in the tube

(e) Negative: No color development or slightly pink color.

The test culture was inoculated into peptone water and incubated at 37°C for 48 h. One ml of Kovacs reagent was added to the tube. The appearance of a pink color in the reagent layer within a minute indicated positive reaction.

3.2.11.2.3 Methyl red (MR) test

This test was performed to differentiate Enterobacteria. Some Enterobacteria when cultured in buffered glucose peptone water, ferment glucose to produce sufficient acidity, which gives red color with methyl red indicator (PH range: 4.4-6.2, Color change: red yellow) by (Cheesbrough, 1985).

Procedure

(a) Sterile MR-VP broth was inoculated with the test organism and following incubation at 370 c for 24 hours.

(b) Few drops of methyl red solution were added.

Observations:

A distinct red color indicated MR positive test
Yellow or orange color indicated a negative result.

3.2.11.2.4 Voges Proskauer (VP) test

The test organisms were cultured in glucose phosphate peptone water for 24 hours. Acetone formed was converted to diacetyl. It was converted to a pink compound by the action of creatine. This test was used to assist in the differentiation of Enterobacteria such as *Vibrio cholerae*, *Klebsiella* spp and some strains of Enterobacter (Cheesbrough,1985).

Procedure

Sterile MR-VP broth was inoculated with the test organism and following incubation at 37° C for 24 hours.
After incubation, 5 drops of napthol solution and 5 drops of KOH solution were added.

Observations: The development of a bright red or pink-red color was recorded as a positive Result.

3.2.11.2.5 Citrate utilization test

The test organism was cultured in a medium containing sodium citrate, an ammonium salt and bromothymol blue indicator. The organisms use citrate (the only source of28

nitrogen). The citrate utilization is followed by alkaline reaction (change of the color form light to blue) and growth in the medium was indicated by appearance of turbidity. This test was performed in the identification of Enterobacteria by (Cheesbrough, 1985).

Procedure

A loop of bacteria was spread across the surface of the agar.
Kept the tubes at 370C for 24 hours for incubation.
Examined the tubes for the result.

Observations:

Positive: - produce blue color,
Negative: - no color

3.2.11.2.6 MIU (Motility Indole Urease) test

MIU is a semisolid medium designed for detection in Enterobacteriaceae of urease activity, motility, and indole production by Cheesbrough, 1985).

Procedure

Inoculate tubes with a pure culture by stabbing the center of the column of medium to greater than half the depth. Incubate tubes for 18-48 hours at 35 ± 2 °C in aerobic atmosphere.

Motility was observed by growth extending from the line of inoculum or diffuse turbidity of the medium. Nonmotile organisms grow only along the line of inoculation.
Urease activity was observed by a change of color to red.
Indole production is indicated by the formation of a pink to red color after the addition of three or four drops of Kovac's reagent to the surface of the medium.

Observations: A negative reaction is indicated by the development of a yellow color. The red color of phenol red in alkaline pH did not interfere because of the acidity of kovac's reagent. By (Cheesbrough, 1985)

3.2.11.2.7 Triple sugar Iron (TSI) agar slant

65 grams TSI agar base powder was mixed in 1000 ml of cold distilled water in a flask and mixed thoroughly, then heated to boiling for dissolving the medium completely

(necessary calculation was done for required number of test tubes).The medium was then sterilized by autoclaving for 15 minutes at 121°c maintaining a pressure of 1.2 kg/.Then 20/10 ml of medium was poured into each sterilized test tubes and allowed to cool and to solidify (kept in horizontal position). After solidification test tube were used for biochemical characterization and incubated at 37°c for 24 hours.

Procedure

A loop of bacteria was spread across the surface of the agar.

A needle of bacteria was inserted (stabbed) into the bottom (butt) of the tube.

Kept the tubes at 37^0C for 24 hours for incubation.

Examined the tubes for the result.

Table 3.1: Triple sugar Iron (TSI) agar slant

Result (Slant/butt	Symbol	Interpretation
Red/yellow	K/A	Glucose fermentation only
Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation
Red/Red	K/K	No fermentation
Yellow/Yellow with bubbles	A/A, G	Glucose and lactose and/or sucrose fermentation; Gas produced
Red/Yellow with bubbles	K/A, G	Glucose fermentation only; Gas produced
Red/Yellow with bubbles and black precipitation	K/A, G H_2S	Glucose fermentation only; Gas produced H2S Produced
Red/Yellow with black precipitation	K/A, H_2S	Glucose fermentation only; H_2S Produced
Yellow/Yellow with black precipitation	A/A, H_2S	Glucose and lactose and/or sucrose fermentation; H_2S Produced
No Change/ No Change	NC/NC	No fermentation

Note: A=acid production: K= alkaline reaction: G=gas production: H_2S= Hydrogen sulfide production.

3.2.11.2.8 Antibiotic sensitivity tests

Antibiotic sensitivity assay of isolated bacteria, Bacterial susceptibility to anti-microbial agent was determined in vitro by using the standardized agar disc-diffusion method known as the Kirby Bauer (K-B), Labeled the covers of each of the agar plates with name of the test organisms was inoculated.

(a) Using sterile technique, inoculated all agar plates with their respective test organisms as follow:

Dipped a sterile cotton swab into a well-mixed saline test culture and removed excess inoculums by pressing the saturated swab against the inner wall of the culture tube.

Using the swab streaked the entire agar surface horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface.

(b) Allowed all culture plates to dry for about 5 minutes.

(c) Distributed the individual antibiotic discs at equal distance with forceps dipped in alcohol and flamed.

(d) Gently pressed each disc down with the wooden end of the cotton swab or sterile forceps to ensure that the discs adhered to the surface of the agar.

(e) The plates were then inverted and incubated at 37^0 C for 24 hours.

(f) After incubation, the plates were examined and the diameter of the zones of complete inhibition was measured in mm.

(g) The zone diameter for individual anti-microbial agents was used to determine susceptible, intermediate, and resistant categories by referring to an interpreting table

Table 3.2: Antimicrobial agents with their disc-concentration

Sl. No	Antimicrobial Agents	Symbol	Disc concentration (µg/disc)
1	Amoxicillin	AMX	30
2	Ampicillin	AMP	25
3	Ciprofloxacin	CIP	5
4	Gentamicin	GEN	10
5	Levofloxacin	LE	5
6	Erythromycin	E	30
7	Azithromycin	AZM	30
8	Chloramphenicol	C	30
9	Colistin	CL	10
10	Ceftriaxone	CTR	30

Source: CLSI- 2013

3.2.12 Reading Plates and Interpreting Results

After 24 hours of incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones oh inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies were apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, nonreflecting background and zones are measured in millimeter (mm) from the upper surface of the agar illuminated with reflected light, with the cover removed (EUCAST, 2015).

Table 3.3: Represent interpretation result of antibiotic sensitivity test.

Antimicrobial agent active against bacteria (Disc Code)	Potency (µg/disc)	Zone Diameter Nearest Whole mm		
		Resistant ≤	Intermediate	Susceptible ≥
Gentamicin (GEN) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Staphylococcus* spp.	10 µg/disc	12	13-14	15
Amoxicillin (AMX) *Entrobacteriaceae* *Staphylococcus* spp.	30 µg/disc	13	14-17	18
Ceftriaxone (CTR) Entrobacteriaceae Staphylococcus spp	30 µg/disc	13	14-20	21
Azithromycin (AZM) Staphylococcus spp. Streptococcus spp.	30 µg/disc	13	14-17	18
Erythromycin (E) Staphylococcus spp. Enterococcus spp	15 µg/disc	13	14-22	23
Ampicillin(AMP) Entrobacteriaceae. Acinetobacter spp. Pseudomonas aeruginosa	25 µg/disc	28	-	30
Colistin (CL) Streptomycin (S) Entrobacteriaceae	10 µg/disc	12	-	14
Chloramphenicol(C) Entrobacteriaceae Staphylococcus spp Streptococcus spp.	30µg/disc	13	14-17	18
Ciprofloxacin(CIP) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Acinetobacter* spp. *Staphylococcus* spp**.**	5µg/disc	15	16-20	21
Levofloxacin(LE) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Staphylococcus* spp	5µg/disc	20	-	21

3.2.13 Maintenance of stock culture

After completion of characterization of bacterial pathogens it was necessary to preserve the isolated organisms for longer periods. For this purpose, pure culture of the isolated *E. coli and vibrio* spp were stored in 10% glycerin and used as stock culture.prior to use glycerin must be autoclaved. The equal volume of 10% glycerin and bacterial culture were mixed and sealed with paraffin wax and stored at 37°C. The isolated organisms were given code name for convenience.

3.2.14 Polymerase Chain Reaction (PCR)

Lane 1: 100 bp DNA size marker, lane 2, 3, 4, 5: genomic DNA of *E. coli* and lane 6: negative control without DNA: PCR amplification,sequencing of 16 rRNA genes with universal primers and analysis of *E.Coli* and *vibrio* spp. Result of PCR targeting 16S rRNA gene for the identification of *E. coli and Vibrio* spp

CHAPTER 4

RESULTS

Result of total viable count, Most probable number,cultural test, staining characteristics, biochemical test and antibiotic sensitivity test, including percentage of isolated bacteria are presented in different tables and described below under the following heading:-

4.1 Total Viable Count (TVC/ in the form of CFU/ml) in water of different sources

Table 4.1: TVC of drinking water obtained from different tubewell of human

Type	Place	Sample no.	TVC	Log 10	TVC Mean
Tubewell water	Sadar	T1	2.7×10^3	3.43	2.55×10^3
		T2	1.7×10^3	3.23	
		T3	2.5×10^3	3.40	
		T4	5.0×10^3	3.70	
		T5	2.5×10^3	3.40	
		T6	1.0×10^3	3.0	
	Birgonj	T1	7.5×10^3	3.70	3.11×10^3
		T2	1.1×10^3	3.40	
		T3	1.5×10^3	3.0	
		T4	1.5×10^3	3.87	
		T5	5.0×10^3	3.04	
		T6	1.5×10^3	3.17	
	Kaharol	T1	1.1×10^3	3.70	2.85×10^3
		T2	1.5×10^3	3.40	
		T3	5.5×10^3	3.0	
		T4	4.5×10^3	3.87	
		T5	2.0×10^3	3.04	
		T6	2.5×10^3	3.17	
	Birol	T1	1.1×10^3	3.70	2.88×10^3
		T2	1.5×10^3	3.40	
		T3	5.5×10^3	3.0	
		T4	4.5×10^3	3.87	
		T5	2.0×10^3	3.04	
		T6	2.5×10^3	3.17	
	Chirirbandar	T1	4.2×10^3	3.70	3.20×10^3
		T2	3.5×10^3	3.40	
		T3	2.5×10^3	3.0	
		T4	3.0×10^3	3.87	
		T5	2.5×10^3	3.04	
		T6	3.5×10^3	3.17	

Mean TVC: Sadar 2.55×10^3, Birgonj 3.11×10^3, Kaharol 2.85×10^3 , Birol 2.88×10^3 and Chirirbandar 3.20×10^3 CFU mL^{-1}

Table 4.2: TVC of drinking water obtained from tubewell of dairy farm

Type	Place	Sample no.	TVC	Log 10	Mean TVC
Tubewell	Sadar	F1	2.7×10^3	3.43	2.3×10^3
		F2	1.7×10^3	3.23	
		F3	2.5×10^3	3.40	
	Birgonj	F1	7.5×10^3	3.87	3.6×10^3
		F2	1.1×10^3	3.04	
		F3	1.5×10^3	3.17	
	Kaharol	F1	1.1×10^3	3.04	2.7×10^3
		F2	1.5×10^3	3.17	
		F3	5.5×10^3	3.74	
	Birol	F1	4.2×10^3	3.62	3.2×10^3
		F2	2.5×10^3	3.40	
		F3	3.5×10^3	3.54	
	Chirirbandar	F1	4.2×10^3	3.62	3.4×10^3
		F2	3.5×10^3	3.54	
		F3	2.5×10^3	3.40	

Mean TVC : Sadar 2.3×10^{3}, Birgonj 3.6×10^{3}, Kaharol2.7×10^{3}, Birol 3.2×10^3 and Chirirbandar 3.4×10^3 CFU mL^{-1}

Table 4.3: TVC of drinking water obtained from manger of dairy farm

Type	Place	Sample no.	TVC	Log10	Mean TVC
Manger	Sadar	F1	4.80×10^7	7.681	4.66×10^7
		F2	4.4×10^7	7.643	
		F3	4.8×10^7	7.681	
	Birgonj	F1	2.8×10^7	7.447	2.0×10^7
		F2	4.4×10^5	5.643	
		F3	3.2×10^7	7.505	
	Kaharol	F1	4.00×10^6	6.602	2.8×10^7
		F2	3.60×10^7	7.556	
		F3	4.40×10^7	7.643	
	Birol	F1	2.80×10^5	5.447	2.8×10^7
		F2	4.80×10^7	7.681	
		F3	3.60×10^7	7.556	
	Chirirbandar	F1	2.60×10^7	7.415	4.2×10^7
		F2	5.20×10^7	7.716	
		F3	4.80×10^7	7.681	

Mean TVC: Sadar 4.66×10^{7}, Birgonj 2.0×10^{7}, Kaharol 2.8×10^7, Birol 2.8×10^7 and Chirirbandar 4.2×10^7 CFU mL^{-1}

Table 4.4: TVC of drinking water obtained from tubewell of Poultry farm

Type	Place	Sample no.	TVC	Log10	Mean TVC
Tubewell	Sadar	P1	5.0×10^3	3.70	2.8×10^3
		P2	2.5×10^3	3.40	
		P3	1.0×10^3	3.0	
	Birgonj	P1	1.5×10^3	3.17	2.67×10^3
		P2	5.0×10^3	3.70	
		P3	1.5×10^3	3.17	
	Kaharol	P1	4.5×10^3	3.65	3.0×10^3
		P2	2.0×10^3	3.30	
		P3	2.5×10^3	3.40	
	Birol	P1	3.7×10^3	3.56	2.57×10^3
		P2	2.5×10^3	3.40	
		P3	1.5×10^3	3.17	
	Chirirbandar	P1	3.0×10^3	3.47	3.0×10^3
		P2	2.5×10^3	3.40	
		P3	3.5×10^3	3.54	

Mean TVC :Sadar 2.8×10^{3}, Birgonj 2.67×10^{3}, Kaharol 3.0×10^{3}, Birol 2.57×10^3 and Chirirbandar 3.0×10^3 CFU mL^{-1}

Table 4.5: TVC of drinking water obtained from waterer of Poultry farm

Type	Place	Sample no.	TVC	Log10	Mean TVC
Waterer	Sadar	P1	4.00×10^7	7.602	4.2×10^7
		P2	4.20×10^7	7.623	
		P3	4.40×10^7	7.643	
	Birgonj	P1	3.60×10^7	7.556	2.00×10^7
		P2	3.60×10^5	5.556	
		P3	2.40×10^7	7.380	
	Kaharol	P1	3.20×10^6	6.505	3.25×10^7
		P2	2.40×10^7	7.380	
		P3	1.60×10^7	7.204	
	Birol	P1	2.00×10^5	5.301	1.00×10^7
		P2	1.20×10^7	7.079	
		P3	1.80×10^7	7.255	
	Chirirbandar	P1	1.60×10^7	7.204	2.00×10^7
		P2	2.40×10^7	7.380	
		P3	2.00×10^7	7.301	

Mean TVC : Sadar 4.2×10^7 , Birgonj 2.00×10^7 , Kaharol 3.25×10^7 , Birol 1.00×10^7 and Chirirbandar $2.00\times10^7 10^3$ CFU mL^{-1}

4.2 Most Probable Number (MPN/100ml) in drinking water of different sources

Table 4.6: MPN of drinking water obtained from different tubewell of human

Type	Place	Sample no.	MPN	Mean MPN
Tubewell water	Sadar	T1	1	2.16
		T2	2	
		T3	2	
		T4	4	
		T5	2	
		T6	2	
	Birgonj	T1	4	2.16
		T2	1	
		T3	2	
		T4	2	
		T5	2	
		T6	2	
	Kaharol	T1	2	2.5
		T2	2	
		T3	4	
		T4	1	
		T5	2	
		T6	4	
	Birol	T1	0	1.83
		T2	4	
		T3	2	
		T4	1	
		T5	2	
		T6	2	
	Chirirbandar	T1	2	2.33
		T2	4	
		T3	2	
		T4	2	
		T5	2	
		T6	2	

Mean MPN: Sadar 2.16,Birgonj 2.16, Kaharol 2.5, Birol 1.83 and Chirirbandar 2.33 colliforms/100ml water

Table 4.7: MPN of drinking water obtained from different tubewell and manger of dairy farm

Type	Place	Sample no.	MPN	Mean MPN
Tubewell	Sadar	F1	1	1.66
		F2	2	
		F3	2	
	Birgonj	F1	4	2.33
		F2	1	
		F3	2	
	Kaharol	F1	2	2.66
		F2	2	
		F3	4	
	Birol	F1	0	2.00
		F2	4	
		F3	2	
	Chirirbandar	F1	2	2.66
		F2	4	
		F3	2	
Manger	Sadar	F1	17	14.00
		F2	14	
		F3	11	
	Birgonj	F1	14	17.33
		F2	17	
		F3	21	
	Kaharol	F1	14	14.00
		F2	11	
		F3	17	
	Birol	F1	21	16.33
		F2	17	
		F3	11	
	Chirirbandar	F1	11	15.33
		F2	21	
		F3	14	

Mean MPN:Tubewell water:Sadar 1.66, Birgonj 2.33, Kaharol 2.66, Birol 2.00 and Chirirbandar2.66 colliforms/100ml water

Mean MPN:Manger water:Sadar 14.00, Birgonj 17.33, Kaharol 14.00, Birol 16.33 and Chirirbandar 15.33 colliforms/100ml water

Table 4.8: MPN of drinking water obtained from different tubewell and waterer of Poultry farm

Type	Place	Sample no.	MPN	Mean MPN
Tubewell	Sadar	P1	1	2.66
		P2	2	
		P3	2	
	Birgonj	P1	2	2.00
		P2	2	
		P3	2	
	Kaharol	P1	1	2.33
		P2	2	
		P3	4	
	Birol	P1	1	1.66
		P2	2	
		P3	2	
	Chirirbandar	P1	2	2.00
		P2	2	
		P3	2	
Waterer	Sadar	P1	11	12.33
		P2	9	
		P3	17	
	Birgonj	P1	9	9.66
		P2	11	
		P3	9	
	Kaharol	P1	11	10.33
		P2	11	
		P3	9	
	Birol	P1	14	13.33
		P2	17	
		P3	9	
	Chirirbandar	P1	11	12.33
		P2	9	
		P3	17	

Mean MPN:Sadar 2.66, Birgonj 2.00, Kaharol 2.33, Birol 1.66 and Chirirbandar 2.00 colliforms/100ml water

Mean MPN:Sadar 12.33 Birgonj 9.66 Kaharol 10.33 Birol 13.33 and Chirirbandar 12.33 colliforms/100ml water

4.3 Total Viable Count (TVC/ml) in water of different sources

The table shows that there were no significance difference of TVC among the tubewell water at different upazila in dinajpur district.The highest TVC of tube-well water was in Chirirbandar(3.50±0.09) followed by the lower TVC were in Birol (3.44±0.16), kaharol (3.38±0.27), Sadar (3.36±0.23) and Birganj (3.35±0.34).

Table 4.9: TVC of drinking water obtained from different tubewell for human

Type of sample	Sadar Mean±SD	Birganj Mean±SD	Kaharol Mean±SD	Birol Mean±SD	Chirirbandar Mean±SD	P Value
Tubewell	3.36±0.23	3.35±0.34	3.38±0.27	3.44±0.16	3.50±0.09	0.406

The table shows that there were no significance difference of TVC of tube-well and manger water among the dairy farm at different upazila in dinajpur district.The highest TVC of tube-well water were found in birol (3.52±0.111) and chirirbandar (3.52±0.111) followed by Birganj (3.36±0.446), Sadar (3.35±0.107) and kaharol (3.31±0.372). The highest TVC of manger water was found in Sadar (7.67±0.021) followed by the lower TVC were found in Chirirbandar (7.60±0.164), kaharol (7.27±0.577), Birol (6.90±1.255) and Birganj (6.86±1.058), but there were significance difference of TVC between tubewell and manger water of dairy farm at different upazilla in dinajpur district.

Table 4.10: TVC of drinking water obtained from tubewell and manger (dairy farm)

Type of sample	Sadar Mean±SD	Birganj Mean±SD	Kaharol Mean±SD	Birol Mean±SD	Chirirbandar Mean±SD	P Value	TVC Mean±SD	Mean P value	Level of significance
Tubewell	3.35±0.107	3.36±0.446	3.31±0.372	3.52±0.111	3.52±0.111	0.814	3.41±0.25	0.013	**
Manger	7.67±0.021	6.86±1.058	7.27±0.577	6.90±1.255	7.60±0.164	0.607	7.26±0.75		

The table shows that there were no significance difference of TVC of tubewell and waterer water among the Poultry farm of different upazila in dinajpur district.The highest TVC of tubewel water was Chirirbandar(3.47±0.070) followed by the lower TVC were kaharol (3.45±0.180), birol (3.38±0.196) ,Sadar (3.37±0.351) and Birganj (3.35±0.306).

The highest TVC of waterer water was Sadar (7.62±0.020) followed by the lower TVC were Chirirbandar(7.30±0.088), kaharol (7.03±0.462), Birganj (6.83±1.107) and Birol (6.55±1.080), but there were significance difference of TVC between tube-well and waterer water of poultry farm at different upazilla in dinajpur district

Table 4.11: TVC of drinking water obtained from tubewell and waterer of Poultry farm

Type of sample	Sadar Mean±SD	Birganj Mean±SD	Kaharol Mean±SD	Birol Mean±SD	Chirirbandar Mean±SD	P Value	TVC Mean±SD	Mean P value	Level of significance
Tubewell	3.37±0.351	3.35±0.306	3.45±0.180	3.38±0.196	3.47±0.070	0.958	3.40±0.210	0.013	**
Waterer	7.62±0.020	6.83±1.107	7.03±0.462	6.55±1.080	7.30±0.088	0.456	7.06±0.722		

4.4 Most Probable Number (MPN/100ml) in drinking water of different sources

The table shows that there were no significance difference of MPN among the tubewell at different upazila in dinajpur district. The highest MPN of tube-well water was kaharol (2.50±1.22) followed by the lower MPN were Chirirbandar(2.33±0.81),Sadar (2.16±0.98), Birganj (2.16±0.98) and Birol (1.83±1.32).

Table 4.12: MPN of drinking water obtained from different tubewell of human

Type of sample	Sadar (Mean±SD)	Birganj (Mean±SD)	Kaharol (Mean±SD)	Birol (Mean±SD)	Chirirbandar (Mean±SD)	P Value
Tubewell	2.16±0.98	2.16±0.98	2.50±1.22	1.83±1.32	2.33±0.81	0.867

The table shows that there were no significance difference of MPN of tubewel and manger water among the dairy farm of different upazila in dinajpur district.The highest MPN of tubewel waterer was kaharol (2.67±1.154)followed by chirirbandar (2.66±1.154),birol (2.00±2.00), Birganj (2.33±1.521) and Sadar(1.67±0.577).The highest MPN of manger water was kaharol (12.33±4.16) followed by the lower TVC were Birganj (12.33±2.89), Chirirbandar (11.33±2.51), Sadar (10.33±1.15) and Birol (9.66±1.15) but there were significance difference of MPN between tubewel and manger of dairy farm at differeny upazilla in dinajpur district

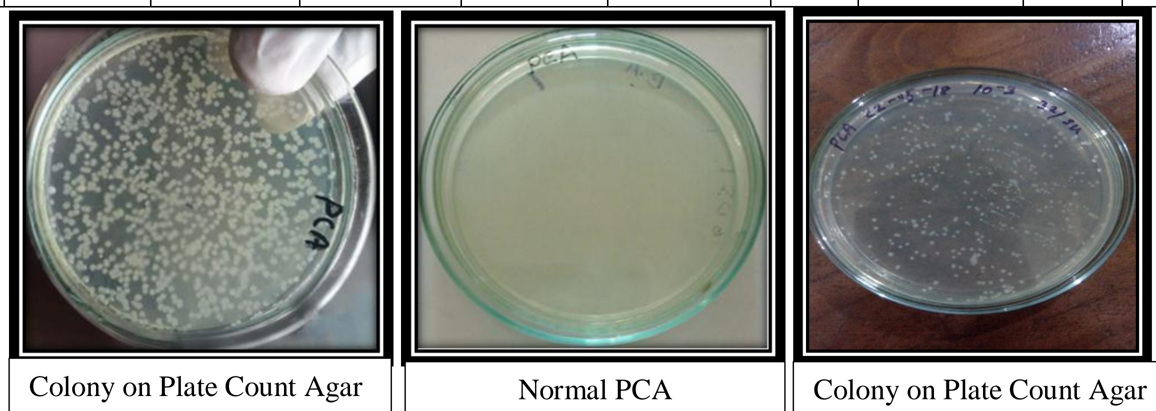
Table 4.13: MPN of drinking water obtained from different tubewell and manger of dairy farm

Type of sample	Sadar Mean±SD	Birganj Mean±SD	Kaharol Mean±SD	Birol Mean±SD	Chirirbandar Mean±SD	P Value	MPN Mean±SD	Mean P value	Level of significance
Tube-well	1.67±0.577	2.33±1.521	2.67±1.154	2.00±2.00	2.66±1.154	0.869	2.266±1.222	0.00	***
Manger	14.00±3.00	17.33±3.51	14.00±3.00	9.66±3.00	16.33±5.03	0.811	15.40±3.68		

The table shows that there were no significance difference of MPN of tubewell and waterer water among the Poultry farm at different upazilla in dinajpur district.The highest MPN of tubewell water was found in Sadar (2.66±1.154) followed by the lower MPN were kaharol (2.33±1.527), Chirirbandar (2.00±0.00), Birganj (2.00±0.00) and Birol (1.66±0.577). The highest MPN of waterer water Birol (13.33±4.04) followed by the lower MPN were Sadar (12.33±4.16), Chirirbandar(12.33±4.16), kaharol (10.33±1.154) and Birganj (9.67±1.154), but there were significance difference of MPN between tubewell and waterer water of poultry farm at different upazilla in dinajpur district.

Table 4.14: MPN of drinking water obtained from different tubewell and waterer of Poultry farm

Type of sample	Sadar Mean±SD	Birganj Mean±SD	Kaharol Mean±SD	Birol Mean±SD	Chirirbandar Mean±SD	P Value	MPN (Mean±SD)	Mean P value	Level of significance
Tubewell	2.66±1.154	2.00±0.00	2.33±1.527	1.66±0.577	2.00±0.00	0.709	2.133±0.833	0.00	**
Waterer	12.33±4.16	9.67±1.154	10.33±1.154	13.33±4.04	12.33±4.16	0.635	11.60±3.11		



Colony on Plate Count Agar | Normal PCA | Colony on Plate Count Agar

Fig. 4.1: Colony Count in Plate Count Agar

4.5 Results of cultural examinations

Cultural characteristics of each type of bacteria isolated from different water sample were studied for the isolatiom, identification of various bacteriological media. The staining property of primary culture of each of the different samples indicated the presence of more than one type of bacteria in the same smear. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different simple and selective solid media for study. The individual cultural characteristics of bacterial isolates are presented in table 4.15. The cultural characteristics of *E. Coli*, *Klebsiella* spp, *Salmonella* spp, *Shigella* spp, *Pseudomonas* spp, *Vibrio* spp and *Staphylococcus* spp exhibited on the media are presented in following figure.

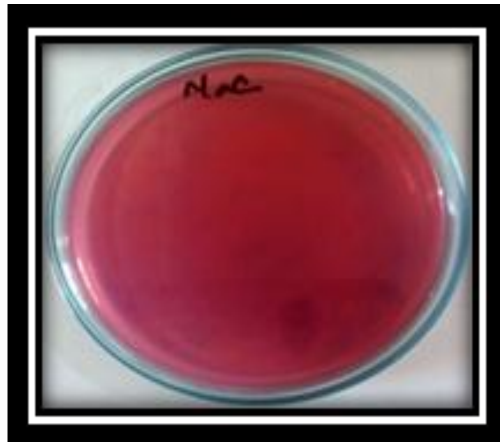
Table 4.15: Cultural characteristics of the bacterial isolates

Sl. No	Suspected case of Bacteria	Name of Media	Cultural Characteristics
01	*Escherichia coli*	EMB agar	Metallic sheen (greenish black) -
02	*Klebsiella* spp.	EMB agar	Brownish pinkish color colony
03	*Salmonella* spp.	SS Agar	Small non-lactose fermented with black center colony
		Brilliant agar	Golden yellowish
04	*Shigella* spp.	SS Agar	Small non-lactose fermented grayish white colony
05	*Staphylococcus* spp.	Agar no. 110	Medium yellowish colony
06	*Pseudomonas* spp.	CET agar	green pigment colonies
07	*Vibrio* spp.	TCBS agar	yellow pigmented colonies

Legends:

AGAR NO. 110, EMB = Eosin Methylene Blue, SS=*Salmonella Shigella,*

TCBS=Thiosulfate-Citrate-Bile salts-Sucrose, CET= Cetrimide



Mackonkey Media (Control)

Mackonkey Media (Growth)

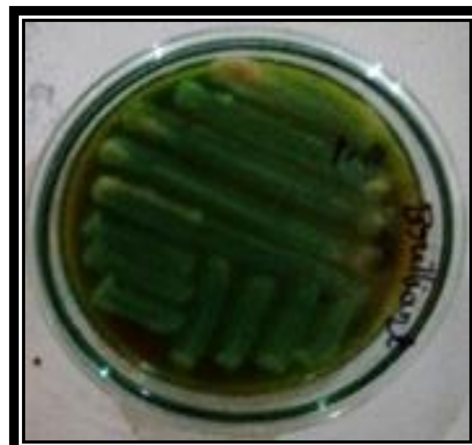
Fig. 4.2: Differential Media

Klebsiella spp

EMB Control

E. coli

Brilliant Green (Control)



Salmonella spp

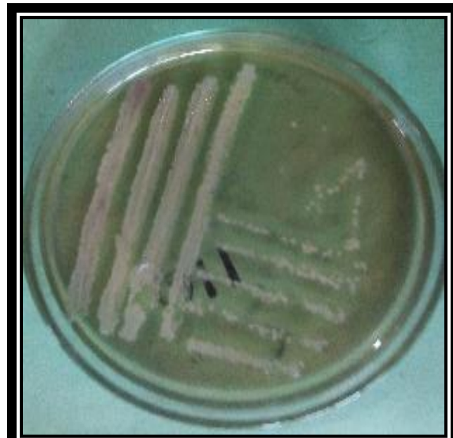
SS Agar (Control)

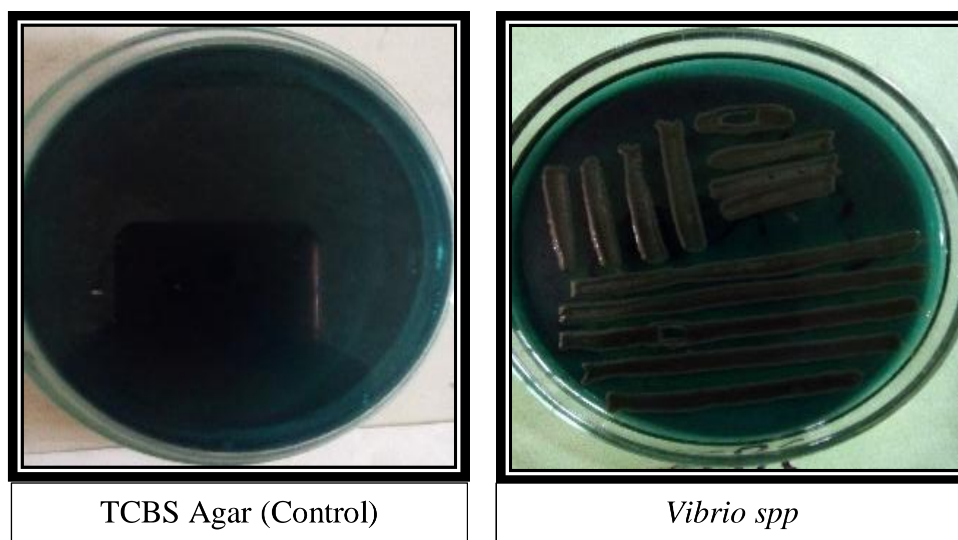
Shigella spp

Cetrimide Agar (Control)

Pseudomonas spp

Agar No. 110 (Control)


Staphyllococcus spp



TCBS Agar (Control)	*Vibrio spp*

Fig. 4.3: Growth of Bacteria on Specific Culture Media

4.6 Results of biochemical tests:

The isolated organisms were confirmed by different biochemical tests. Following **table 11-17** represent the results obtain from different biochemical test for different fish sample.

Table 4.16: Identification of *E. coli* by different biochemical tests

Biochemical test	**Change of the media**	**Results**
MR test	Red color	Positive
VP test	No color change	Negative
Triple sugar iron (TSI) test	Yellow color with gas	S-A, B-A, gas (+), H_2S (-)
MIU test	Turbidity and changing of purple color of media	Positive
Indole test	Pink rose color ring at the top of the media	Positive
Citrate utilization test	No color change, No gas	Negative
Catalase test	No gas bubble	Negative

(**Legends: S**=Slant, B=Butt, A = Acid, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility Indole Urease**,** + = Positive reaction, - = Negative reaction).

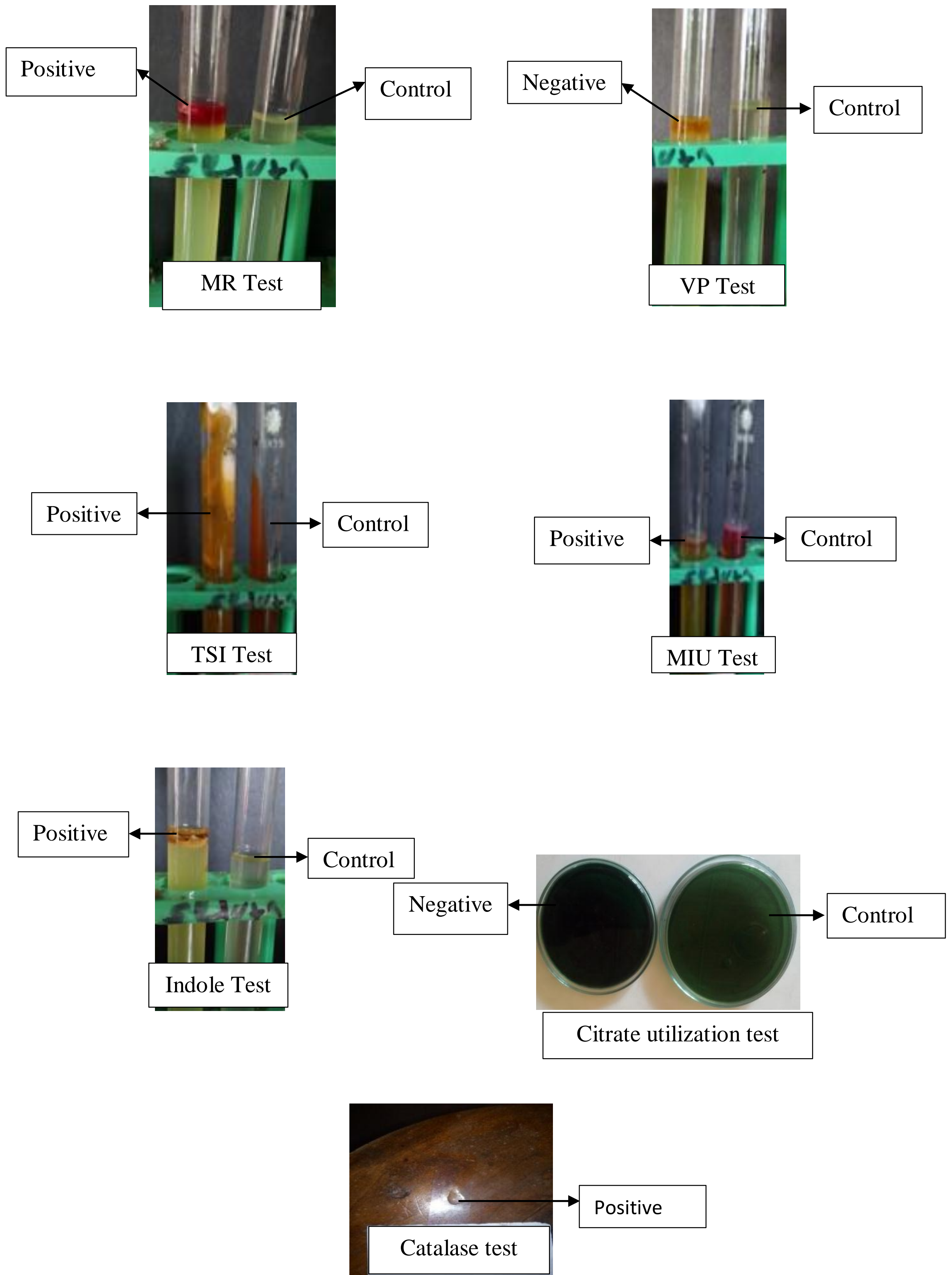


Fig. 4.4: Different biochemical test of *E. coli* spp.

Table 4.17: Identification of *Klebsiella* spp by different biochemical tests

Biochemical test	Change of the media	Results
MR test	Red color	Positive
VP test	No color change	Negative
Triple sugar iron (TSI) test	Yellow color with gas	S-A, B-A, gas (+), H_2S (-)
MIU test	Turbidity and changing of purple color of media	Positive
Indole test	Pink rose color ring at the top of the media	Positive
Citrate utilization test	No color change, No gas	positive
Catalase test	No gas bubble	Negative

(**Legends:** TSI Test, S=Slant, B=Butt, A = Acid, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility Indole Urease**,** + = Positive reaction, - = Negative reaction).

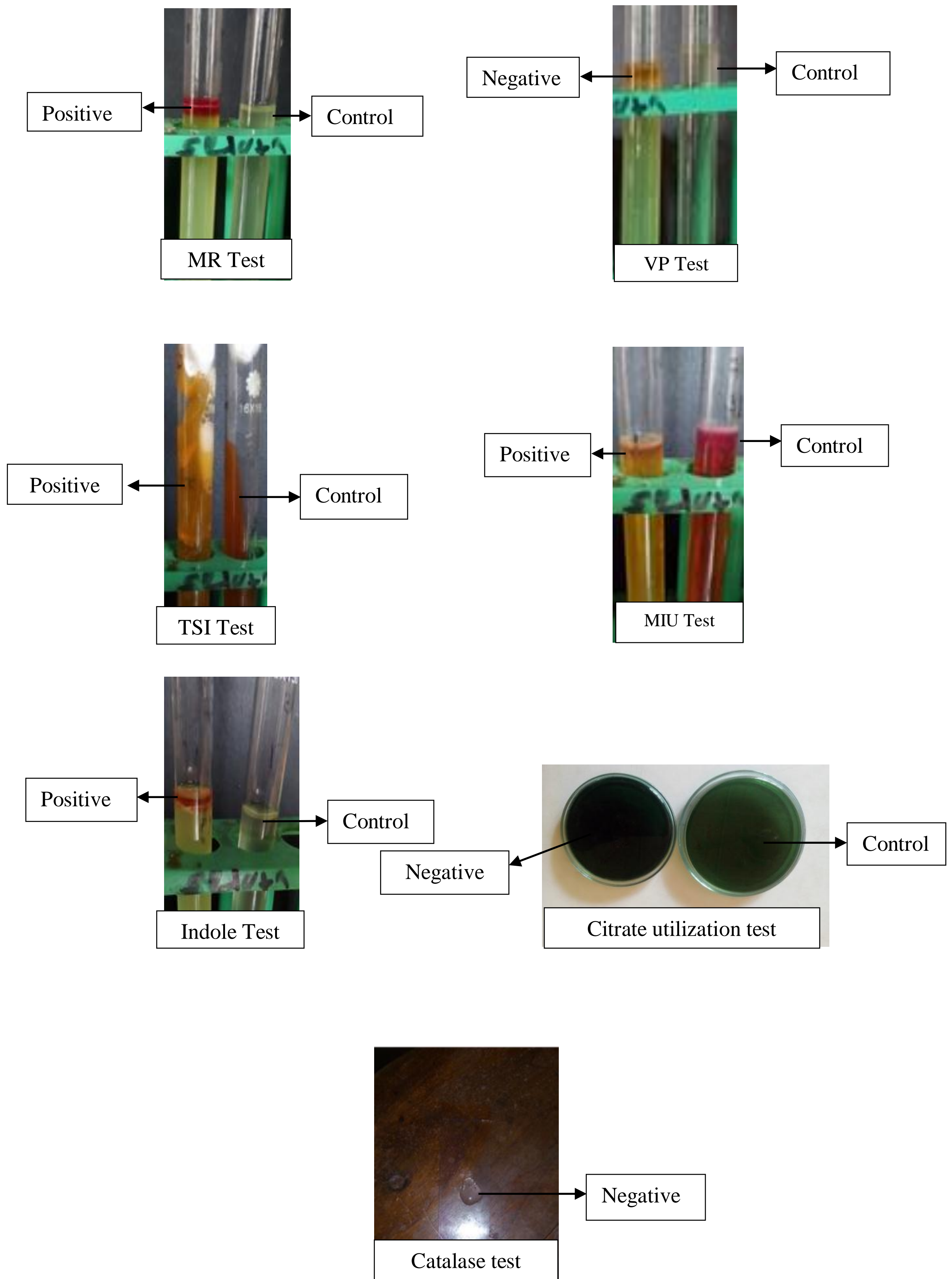


Fig. 4.5: Different biochemical test of *Klebsiella* spp.

Table 4.18: Identification of *Salmonella* spp. by biochemical test

Biochemical test	**Change of the media**	**Results**
MR test	Red color	Positive
VP test	No color change	Negative
Triple sugar iron (TSI) test	S-Red, B-yellow	S-Al, B-A, gas (+), H_2S (+)
MIU test	No turbidity and no changing of color of media	Negative
Indole test	No color change	Negative
Citrate utilization test	No color change	Negative
Catalase test	Gas production	Positive

(**Legends:** TSI Test; **S**=Slant, B=Butt, A = Acid, Al- Alkaline, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility Indole Urease**,** + = Positive reaction, - = Negative reaction).

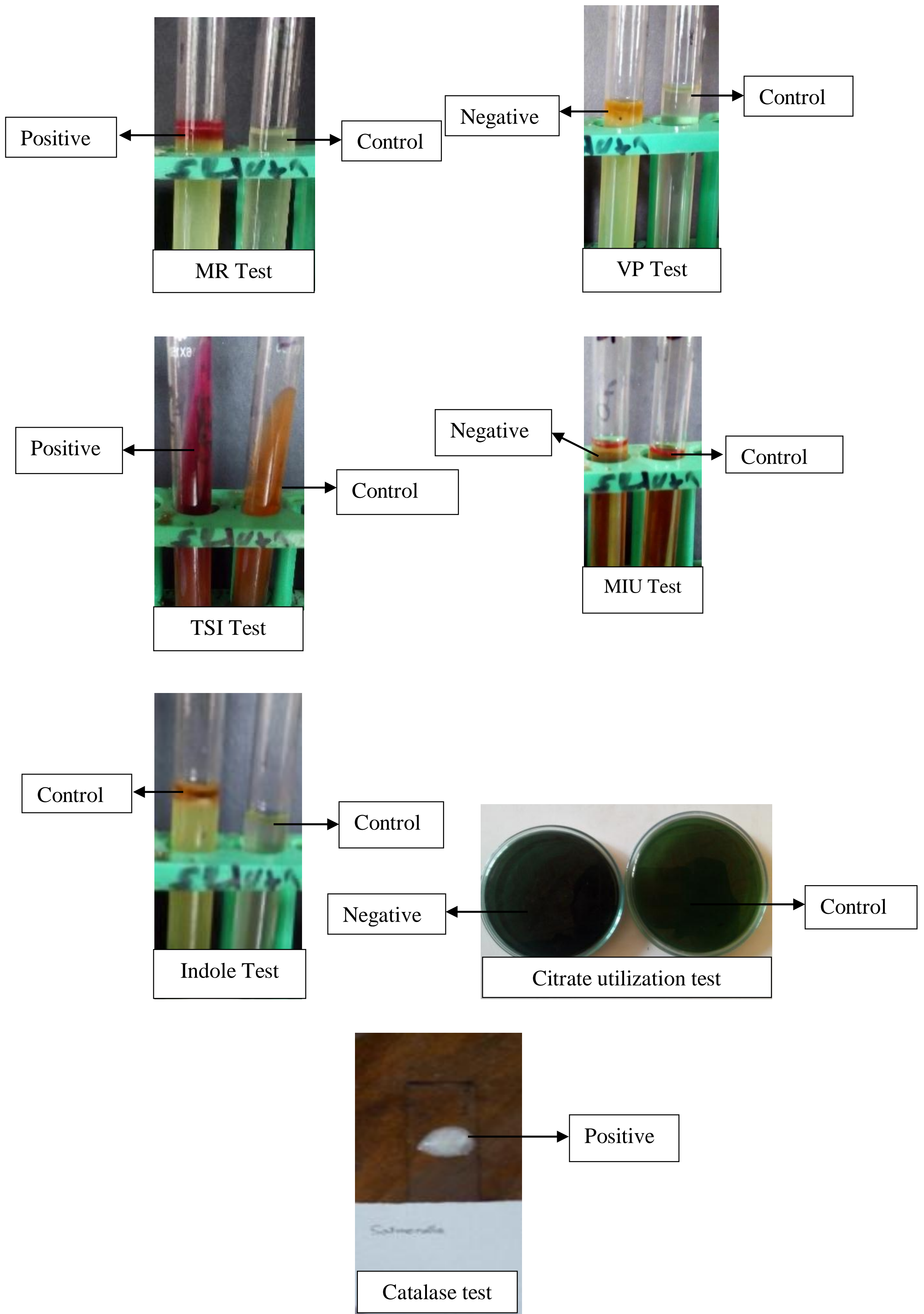


Fig. 4.6: Different biochemical test of *Salmonella* spp.

Table 4.19: Identification of *Shigella* spp. by biochemical test

Biochemical test	**Change of the media**	**Results**
MR test	Red color	Positive
VP test	No color change	Negative
Triple sugar iron (TSI) test	S-Red, B-yellow	S-Al, B-A, gas (+), H_2S (+)
MIU test	No turbidity and no changing of color of media	Negative
Indole test	No color change	Negative
Citrate utilization test	No color change	Negative
Catalase test	Gas production	Positive

(**Legends:** TSI Test; **S**=Slant, B=Butt, A = Acid, Al- Alkaline, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility Indole Urease**,** + = Positive reaction, - = Negative reaction).

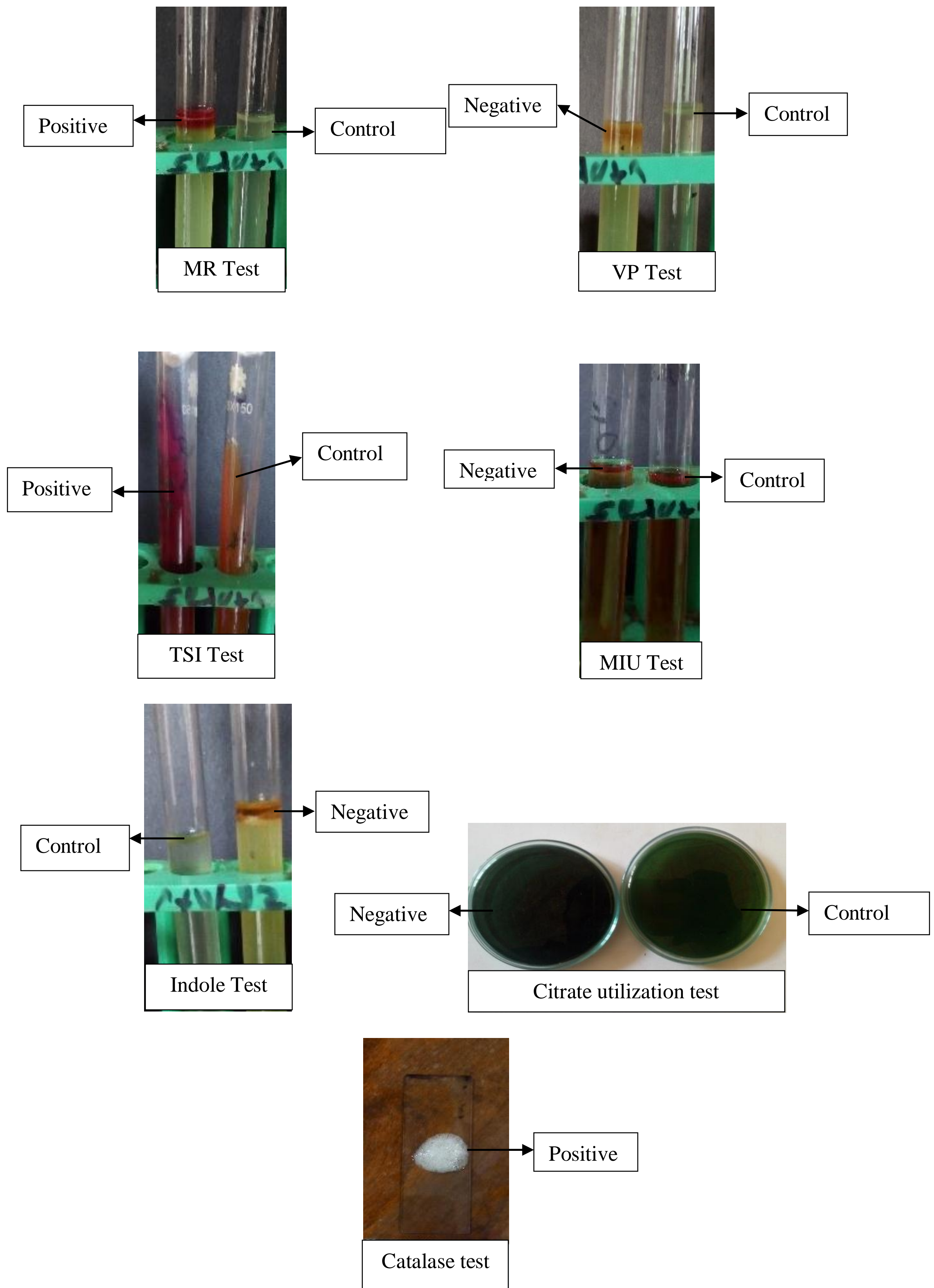


Fig. 4.7: Different biochemical test of *Shigella* spp.

Table 4.20: Identification of *Pseudomonas* spp. by biochemical test

Biochemical test	**Change of the media**	**Results**
MR test	No color change	Negative
VP test	No color change	Negative
Triple sugar iron (TSI) test	S-yellow, B-yellow	S-A, B-A, gas (+), H_2S (-)
MIU test	Turbidity and changing of color of media	Positive
Indole test	No color change	Negative
Citrate utilization test	Prussian blue color	Positive
Catalase test	Bubble produced	Positive

(**Legends:** TSI Test; **S**=Slant, B=Butt, A = Acid, Al- Alkaline, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility indole urease**,** + = Positive reaction, - = Negative reaction).

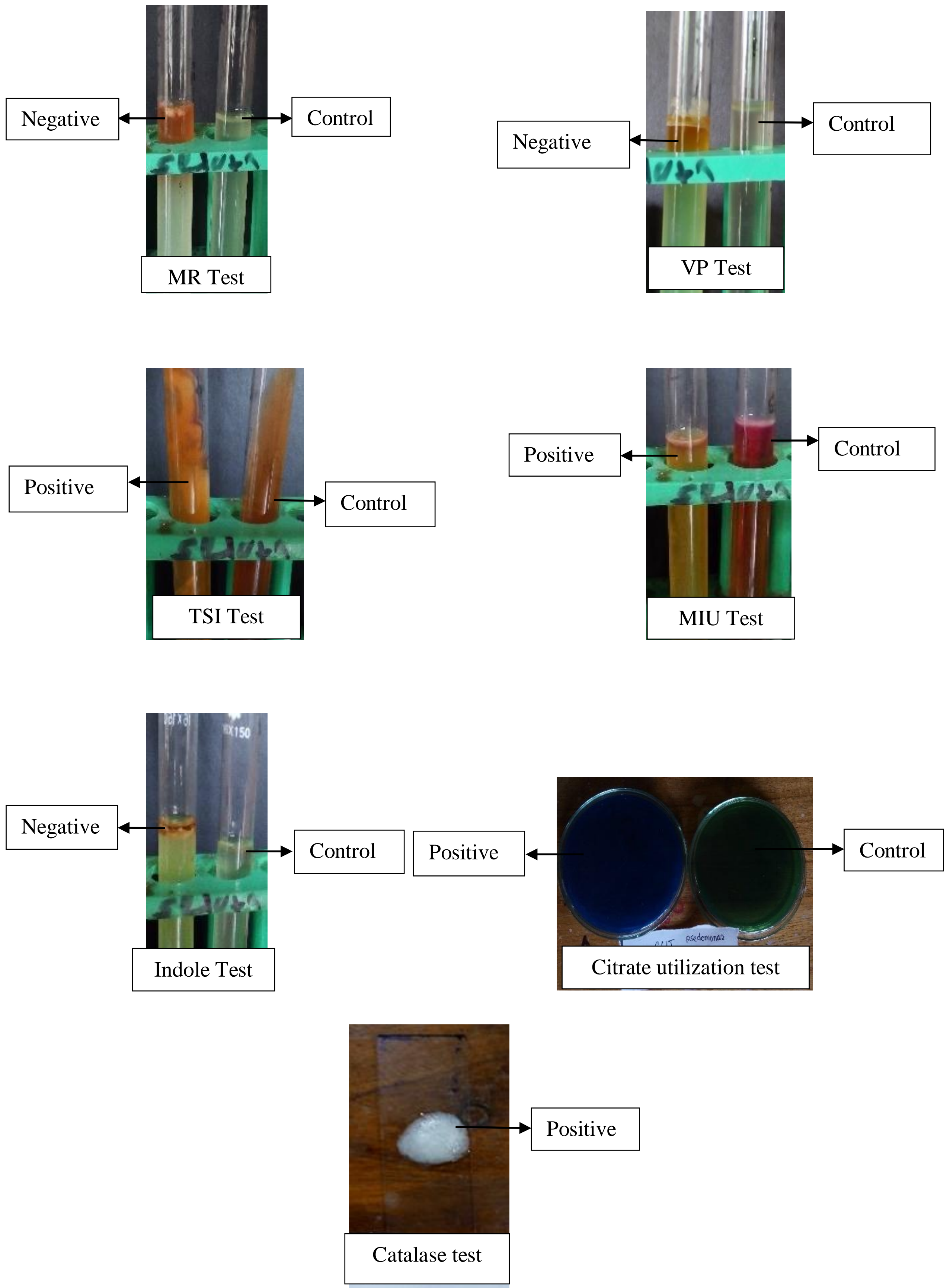


Fig. 4.8: Different biochemical test of *Pseudomonas* spp.

Table 4.21: Identification of *Vibrio* spp. by biochemical test

Biochemical test	**Change of the media**	**Results**
MR test	No color change	Negative
VP test	No color change	Negative
Triple sugar iron (TSI) test	S-yellow, B-yellow	S-A, B-A, gas (+), H_2S (-)
MIU test	Turbidity and changing of color of media	Positive
Indole test	No color change	Positive
Citrate utilization test	Prussian blue color	Positive
Catalase test	No Bubble produced	Negative

(**Legends:** TSI Test; **S**=Slant, B=Butt, A = Acid, Al- Alkaline, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility indole urease**,** + = Positive reaction, - = Negative reaction).

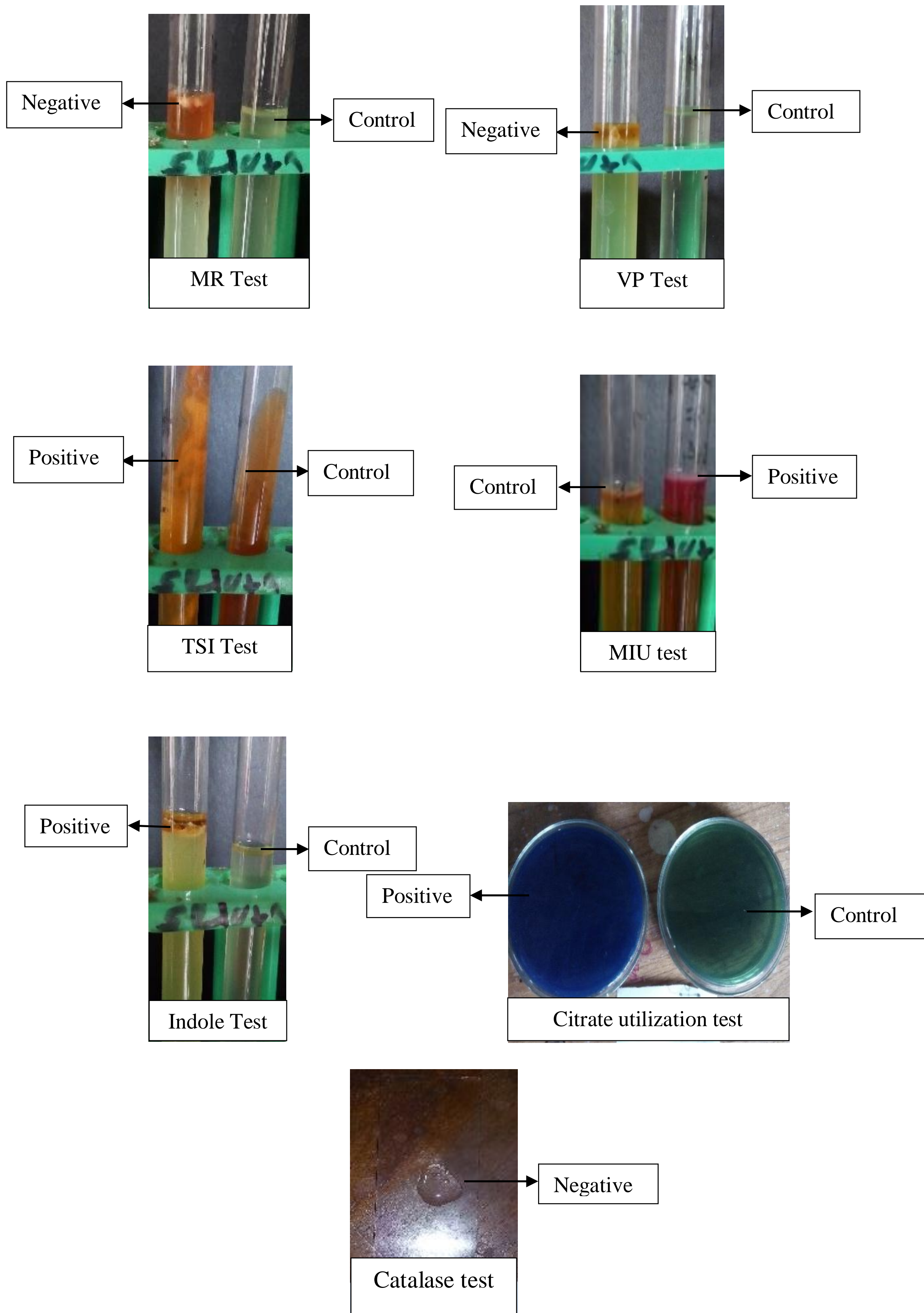


Fig. 4.9: Different biochemical test of *Vibrio* spp.

Table 4.22: Identification of *Staphylococcus* spp. by biochemical test

Biochemical test	**Change of the media**	**Results**
MR test	red color	Positive
VP test	Red color	Positive
Triple sugar iron (TSI) test	S-Red, B-yellow	S-Al, B-A, gas (-), H_2S (-)
MIU test	No turbidity and no changing of color of media	Negative
Indole test	No color change	Negative
Citrate utilization test	No color change	Negative
Catalase test	Bubble produced	Positive

(**Legends:** TSI Test; **S**=Slant, B=Butt, A = Acid, Al- Alkaline, MR = Methyl-Red test, VP = Voges-Proskauer test, MIU= Motility indole urease**,** + = Positive reaction, - = Negative reaction).

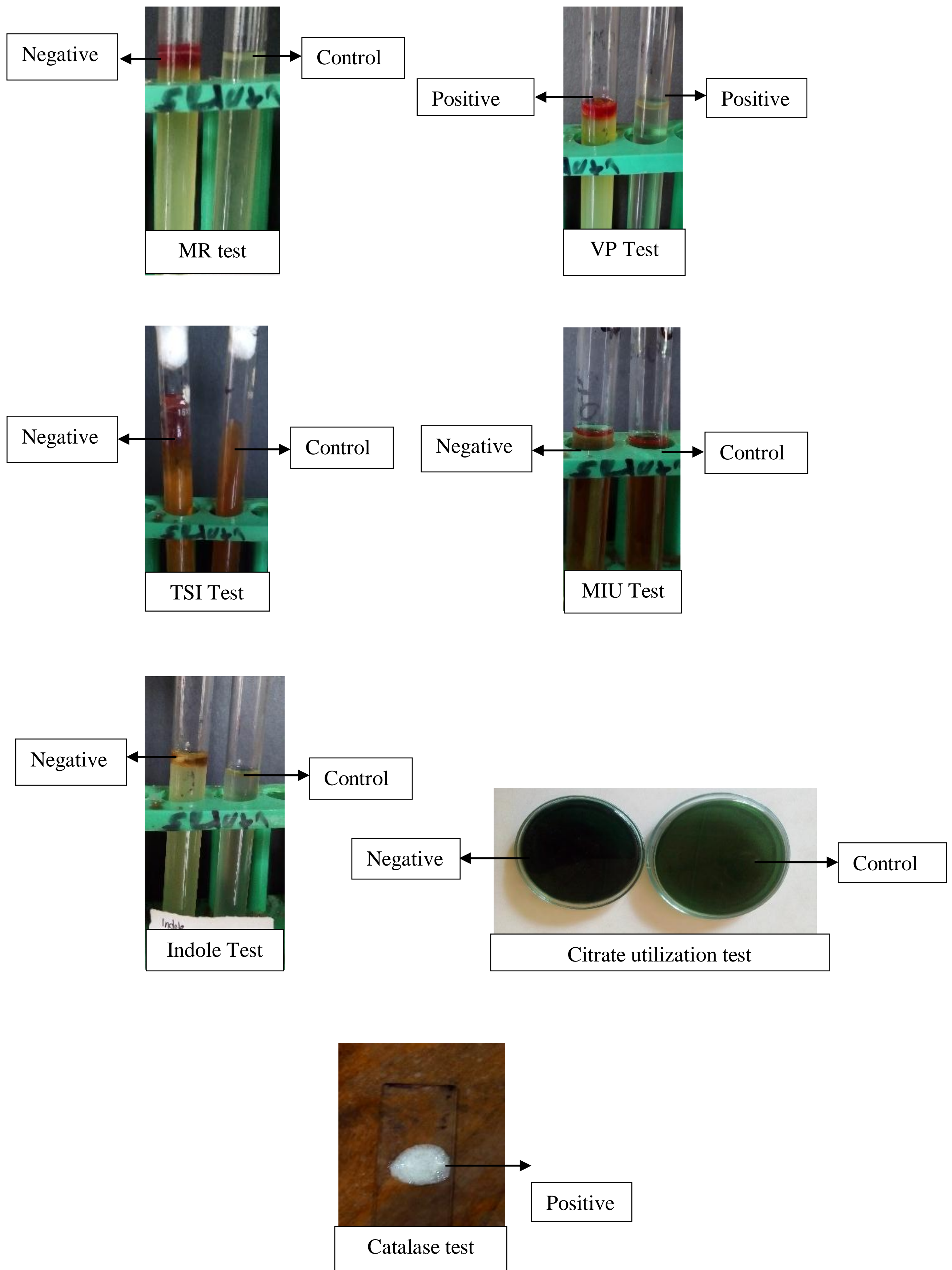


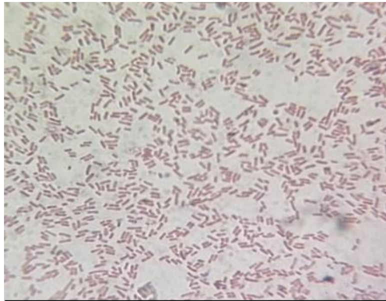
Fig. 4.10: Different biochemical test of *Staphylococcus* spp.

4.7 Result of staining characteristics of Bacterial isolates

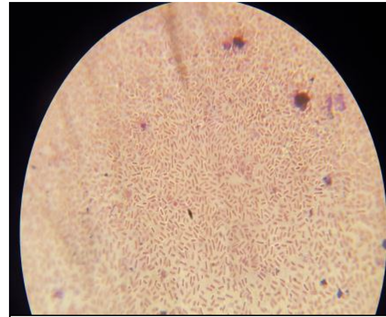
The staining characteristics of the isolated organisms were determined according to Gram's staining technique. Morphological and staining characteristics of bacteria recorded from the swab samples by Gram's staining are presented in Table-10 and following figures.

Table 4.23: Morphological and staining properties of isolated bacteria by Gram's staining

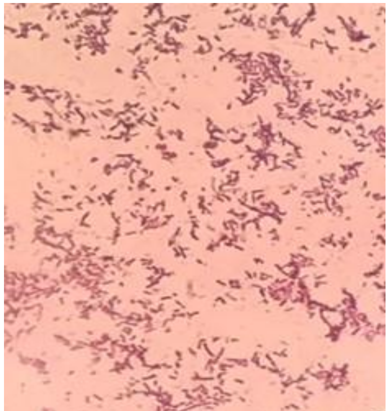
Sl. No	Bacterial isolates	Shape	Arrangement	Gram's Staining character
1.	***Escherichia coli* spp**	Rod in shape	Single, pair or in short chain	Gram (-)
2.	***Klebsiella* spp**	Rod in shape	Single, pairs or cluster	Gram (-)
3.	***Salmonella* spp**	Rod in shape	Single or pair	Gram (-)
4.	***Shigella* spp**	Rod in shape	Single or pair	Gram (-)
5.	***Pseudomonas* spp**	Rod in shape	Arranged in single	Gram (-)
6.	***Vibrio* spp**	Rod in shape	Arranged in single	Gram (-)
7.	***Staphylococcus* spp**	Cocci in shape	Arranged in cluster	Gram (+)



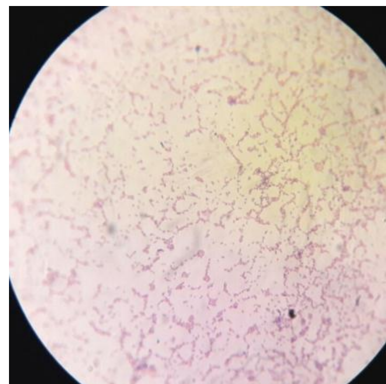
Escherichia coli spp



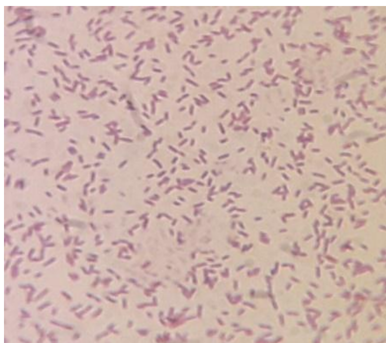
Klebsiella spp



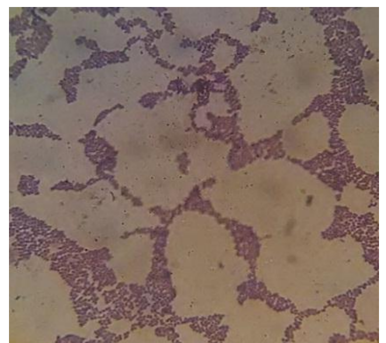
Shigella spp



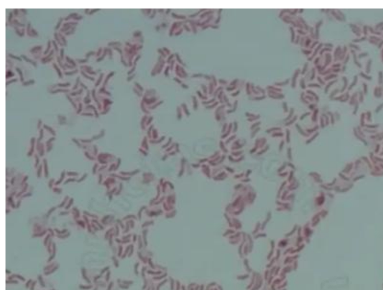
Salmonella spp



Pseudomonas spp



Staphylococcus spp



Vibrio spp

Fig. 4.11: Microscopic view of different bacterial species

4.8 Frequency of Isolated Bacterial Organism

Ninety (90) water sample were collected from different sources; 30 from human water, 30 from dairy farm water and 30 from poultry farm water sources. From ninety (90) water sample *Escherichia coli* was isolated 82(15.95%) highly friquent and followed by *Salmonella* spp 81(15.75%), *Shigella* spp 80(15.56%), *Klebsiella* spp 79(15.36%), *Vibrio* spp 73(14.20%), *Pseudomonas* spp 60(11.67%) and *Staphylococcus* spp 59(11.47%).

Table 4.24: Frequency of Bacteria isolated from water samples

Bacterial species	Number of isolate bacteria					Total (%)
	Human water (30)	Dairy farm (30)		Poultry farm (30)		
	Tubewell water (30)	Tubewell water (15)	Manger (15)	Tubewell water (15)	Waterer (15)	
Escherichia coli	26	13	14	15	14	82(15.95)
Klebsiella spp	24	15	14	12	14	79(15.36)
Salmonella spp	24	13	15	14	15	81(15.75)
Shigella spp	25	12	14	10	15	80(15.56)
Staphylococcus spp	15	8	15	7	14	59(11.47)
Pseudomonas spp	15	7	15	8	15	60(11.67)
Vibrio spp	25	10	15	10	15	73(14.20)

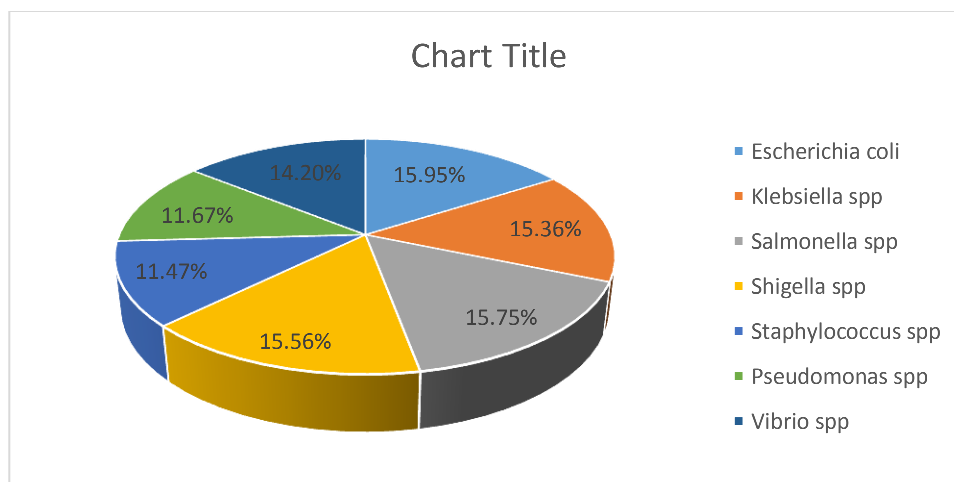


Fig. 4.12: Frequency of Bacteria isolated from water samples

4.9 Results of antibiotics sensitivity tests

Antimicrobial susceptibility testing was performed using Muller-Hinton agar (Mumbai, India) plates as recommended by the Clinical and Laboratory Standards Institute. Seven (7) isolates of *E. coli, Klebsiella* spp*, Salmonella* spp*, shigella* spp*, Staphylococcus* spp*, Pseudomonas* spp. and *Vibrio* spp were subjected to antibiotic sensitivity tests for tap water sample. The results of antibiotics sensitivity tests are presented in Table18,19 and 20.

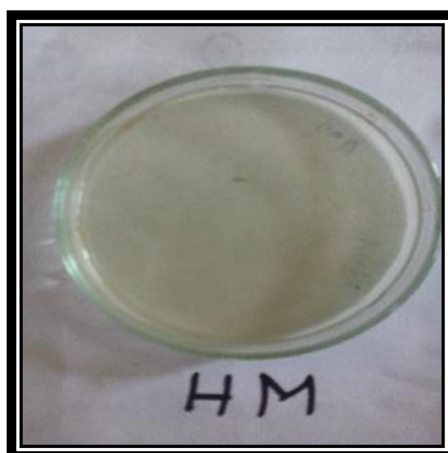
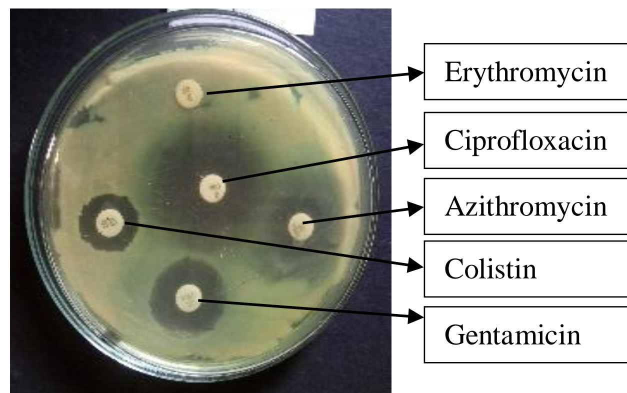


Fig. 4.13: Mueller-Hinton agar

4.9.1 Results of antibiotic sensitivity test of *E. coli* spp.

The results of the antimicrobial sensitivity test by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4.25. Out of 15 *E. coli* isolates, 60% Gentamicin, 20% Ciprofloxacin, 13.33% Levofloxacin, 26.66% Ceftriaxone and 46.66% Chloramphenicol are sussceptible and 53.33% Colistin, 60% Ampicillin, 66.66% Amoxicillin, 60% Erythromycin, 46.66% Azithromycin are resistance.



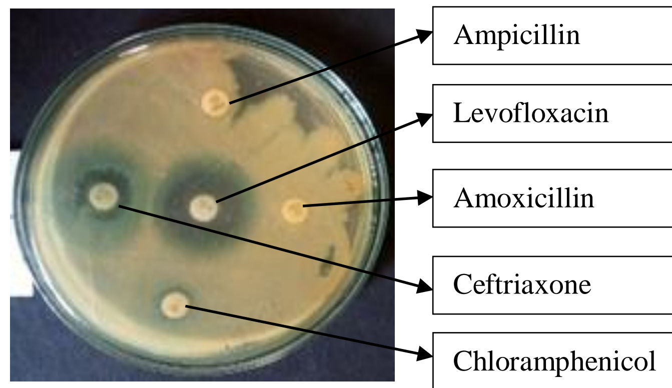


Fig. 4.14: Antibiotic sensitivity test of *E. coli* spp.

Table 4.25: Results of Antimicrobial susceptibility of the isolated *E. coli* spp. from tubewell water

Name of isolates	No. (%)									
E. coli (n=15)	GEN	CIP	C	LE	CTR	CL	AZM	AMP	AMX	E
Susceptible	9(60)	8(53.33)	2(13.33)	8(53.33)	2(13.33)	2(13.33)	2(13.33)	0	0	0
Intermediate	5(33.33)	4(26.66)	6(40)	5(33.33)	9(60)	8(53.33)	6(40)	6(40)	6(33.34)	6(40)
Resistant	1(6.66)	3(20)	7(46.66)	2(13.33)	4(26.66)	5(33.33)	7(46.66)	9(60)	10(66.66	9(60)

Note: GEN=Gentamicin, CIP =Ciprofloxacin, C=Chloramphenicol, LE=Levofloxacin, CTR= Ceftriaxone, CL=Colistin, AZM= Azithromycin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin

I=Intermediate, S=Susceptible and R=Resistance

4.9.2 Results of antibiotic sensitivity test of *Klebsiella* spp.

The results of the antimicrobial sensitivity test by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4.26. Out of 15 *Klebsiella* spp isolates, 60% Gentamicin, 53.33% Ciprofloxacin, 53.33% Levofloxacin, 33.34% Ceftriaxone, 60% Colistin and 60% Azithromycin are sussceptible and 53.33% Ampicillin 60% Amoxicillin, 13.33% Levofloxacin, 66.66% Erythromycin, 13.33% Azithromycin, 53.33% Ampicillin, 60% Amoxicillin, 60% Chloramphenicol are resistance.

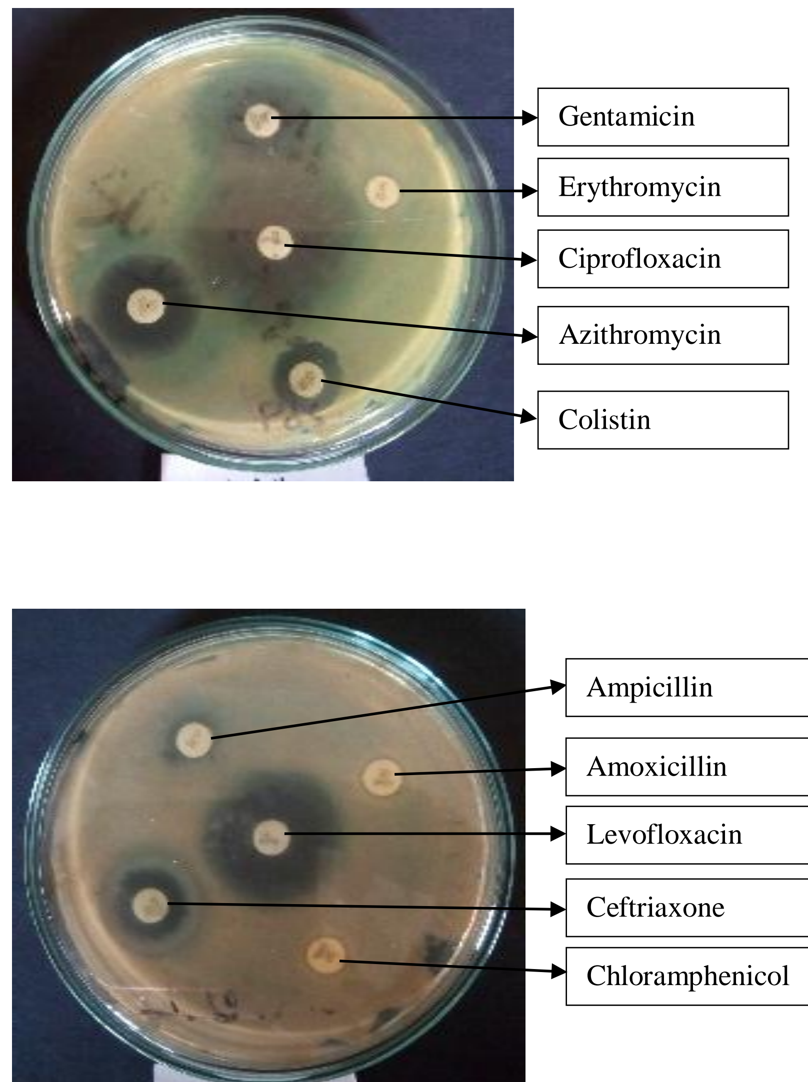


Fig. 4.15: Antibiotic sensitivity test of *Klebsiella* spp.

Table 4.26: Results of Antimicrobial susceptibility of the isolated *Klebsiella* spp tubewell water

Name of isolates	No. (%)									
E. coli (n=15)	GEN	CIP	C	LE	CTR	CL	AZM	AMP	AMX	E
Susceptible	9(60)	8(53.33)	0	8(53.33)	5(33.34)	9(60)	9(60)	0	0	0
Intermediate	6(40)	7(46.66)	6(40)	5(33.33)	10(66.66)	6(40)	4(26.66)	7(46.66)	6(40)	5(33.33)
Resistant	0	0	9(60)	2(13.33)	0	0	2(13.33)	8(53.33)	9(60)	10(66.66)

Note: GEN=Gentamycin, CIP =Ciprofloxacin, C=Chloramphenicol, LE=Levofloxacin, CTR= Ceftriaxone, CL=Colistin, AZM= Azithromycin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin

I=Intermediate, S=Susceptible and R=Resistance

4.9.3 Results of antibiotic sensitivity test of *Salmonella* spp.

The results of the antimicrobial sensitivity test by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4.27. Out of 15 *Salmonella* spp isolates, 60% Gentamicin, 66.66% Ciprofloxacin,13.33% Chloramphenicol, 6.66% Levofloxacin, 33.33% Ceftriaxone, 53.33% Colistin, 53.33% Azithromycin are sussceptible and 53.33% Chloramphenicol, 60% Levofloxacin, 66.66% Ampicillin, 66.66% Amoxicillin, 60% Erythromycin are resistance.

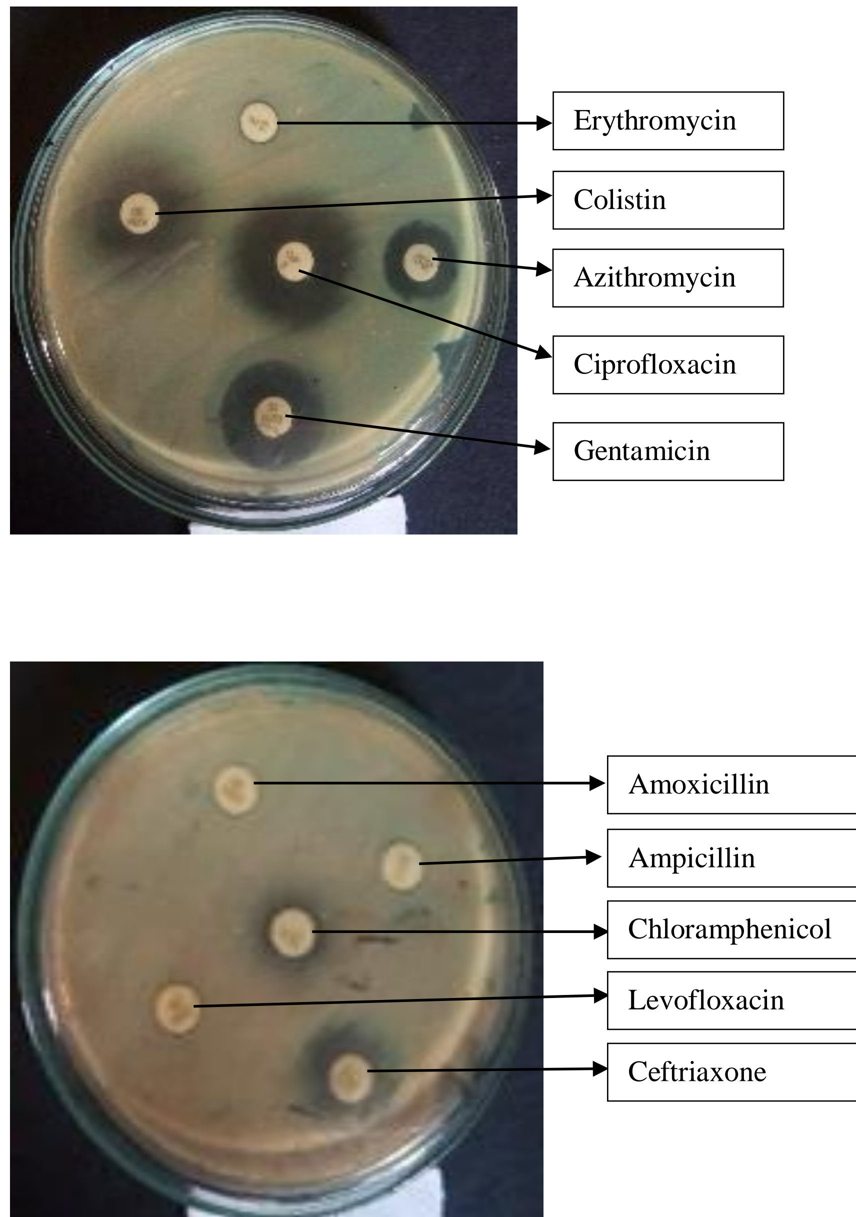


Fig. 4.16: Antibiotic sensitivity test of *Salmonella* spp.

Table 4.27: Results of Antimicrobial susceptibility of the isolated *Salmonella* spp from tubewell water

Name of isolates	No. (%)									
E. coli (n=15)	GEN	CIP	C	LE	CTR	CL	AZM	AMP	AMX	E
Susceptible	9(60)	10(66.66)	2(13.33)	1(6.66)	5(33.33)	8(53.33)	8(53.33)	0	0	0
Intermediate	6(40)	5(33.33)	5(33.33)	5(33.33)	10(66.66)	6(40)	7(46.66)	5(33.33)	0	6(40)
Resistant	0	0	8(53.33)	9(60)	0	1(6.66)	0	10(66,66)	10(66.66)	9(60)

Note: GEN=Gentamicin, CIP =Ciprofloxacin, C=Chloramphenicol, LE=Levofloxacin, CTR= Ceftriaxone, CL=Colistin, AZM= Azithromycin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin

I=Intermediate, S=Susceptible and R=Resistance

4.9.4 Results of antibiotic sensitivity test of *Shigella* spp.

The results of the antimicrobial sensitivity test by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4.28. Out of 15 *Shigella* spp isolates, 66.66%Gentamicin, 66.66% Ciprofloxacin, 73.33% Ceftriaxone, 53.33% Levofloxacin are sussceptible and 60% Chloramphenicol, 33.33% Colistin, 53.33% Azithromycin, 66.66% Ampicillin, 66.66% Amoxicillin, 60% Erythromycin are resistance.

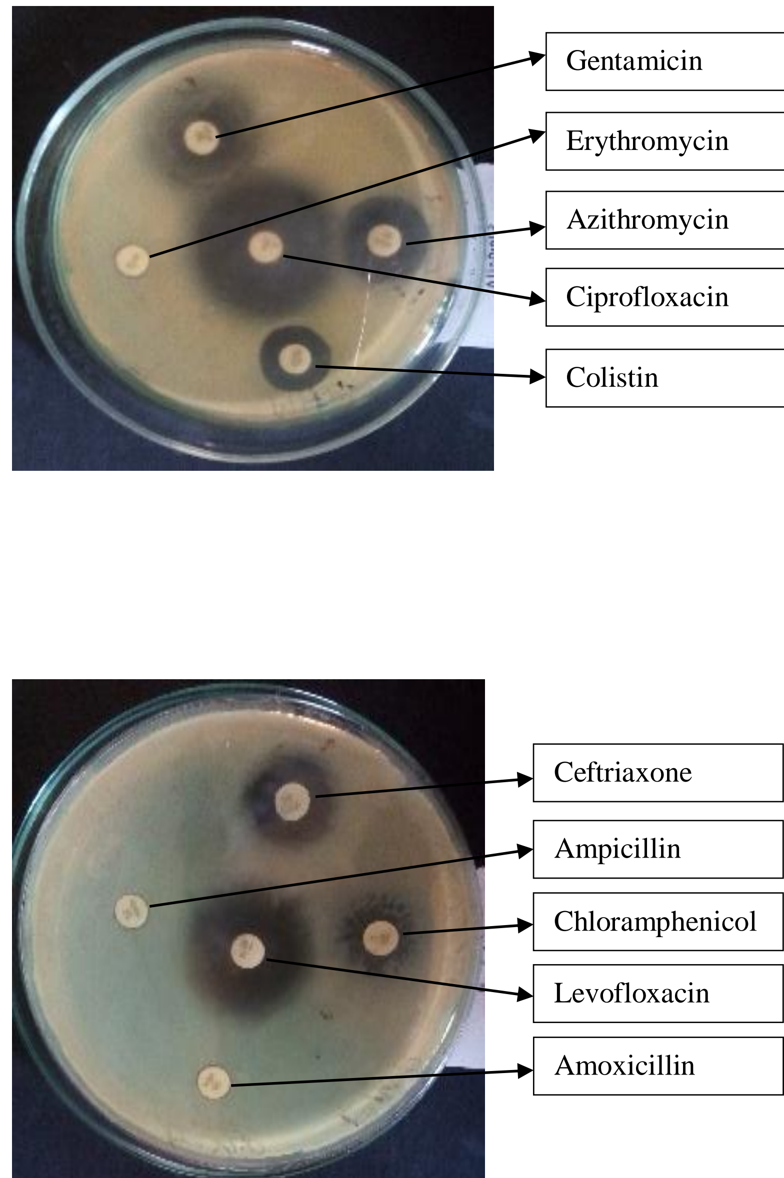


Fig. 4.17: Antibiotic sensitivity test of *Shigella* spp.

Table 4.28: Results of Antimicrobial susceptibility of the isolated *Shigella* spp from tubewell water

Name of isolates	No. (%)									
E. coli (n=15)	GEN	CIP	C	LE	CTR	CL	AZM	AMP	AMX	E
Susceptible	10(66.66)	10(66.66)	2(13.33)	8(53.33)	11(73.33)	1(6.66)	0	1(6.66)	0	0
Intermediate	3(20)	3(20)	4(26.66)	5(33.33)	4(26.66)	9(60)	7(46.66)	4(26.66)	5(33.33)	5(40)
Resistant	2(13.33)	2(13.33)	9(60)	2(13.33)		5(33.33)	8(53.33)	10(66.66)	10(66.66)	9(60)

Note: GEN=Gentamycin, CIP =Ciprofloxacin, C=Chloramphenicol, LE=Levofloxacin, CTR= Ceftriaxone, CL=Colistin, AZM= Azithromycin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin

I=Intermediate, S=Susceptible and R=Resistance

4.9.5 Results of antibiotic sensitivity test of *Pseudomonas* spp.

The results of the antimicrobial sensitivity test by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4.29. Out of 15 *Pseudomonas* spp isolates, 60% Gentamicin, 80% Ciprofloxacin,13.33% Chloramphenicol, 26.66% Colistin, 46.66% Levofloxacin,66.66% Azithromycin are sussceptible and 60% Chloramphenicol, 46.66% Colistin,73.33% Ampicillin, 80% Amoxicillin, 73.33% Erythromycin are resistance.

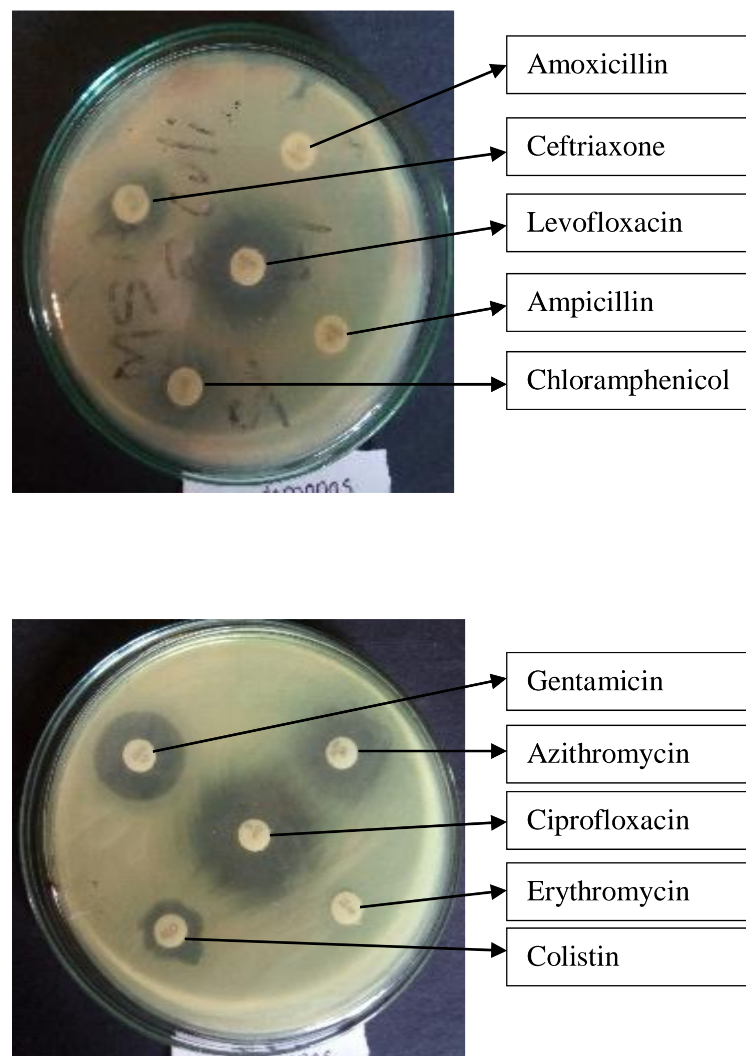


Fig. 4.18: Antibiotic sensitivity test of *Pseudomonas* spp.

Table 4.29: Results of Antimicrobial susceptibility of the isolated *Pseudomonas* spp from tap water.

Name of isolates	No. (%)									
E. coli (n=15)	GEN	CIP	C	LE	CTR	CL	AZM	AMP	AMX	E
Susceptible	9(60)	12(80)	2(13.33)	7(46.66)	2(13.33)	4(26.66)	10(66.66)	0	0	0
Intermediate	4(26.66)	2(13.33)	4(26.66)	5(33.33)	4(26.66)	4(26.66)	3(20)	4(26.66)	3(20)	4(26.66)
Resistant	2(13.33)	1(6.66)	9(60)	3(20)	9(60)	7(46.66)	2(13.33)	11(73.33)	12(80)	11(73.33)

Note: GEN=Gentamicin, CIP =Ciprofloxacin, C=Chloramphenicol, LE=Levofloxacin, CTR= Ceftriaxone, CL=Colistin, AZM= Azithromycin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin

I=Intermediate, S=Susceptible and R=Resistance

4.9.6 Results of antibiotic sensitivity test of *Vibrio* spp.

The results of the antimicrobial sensitivity test by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4.30. Out of 15 *Vibrio* spp isolates, 66.66% Gentamicin, 66.66% Ciprofloxacin,13.33% Chloramphenicol, 46% Colistin, 53.33% Levofloxacin, 73.33% Ceftriaxone are sussceptible and 13.33% Gentamicin, 60% Chloramphenicol, 46.66% Colistin, 13.33% Ciprofloxacin, 13.33% Levofloxacin, 66.66% Ampicillin, 66.66% Amoxicillin, 60% Erythromycin are resistance.

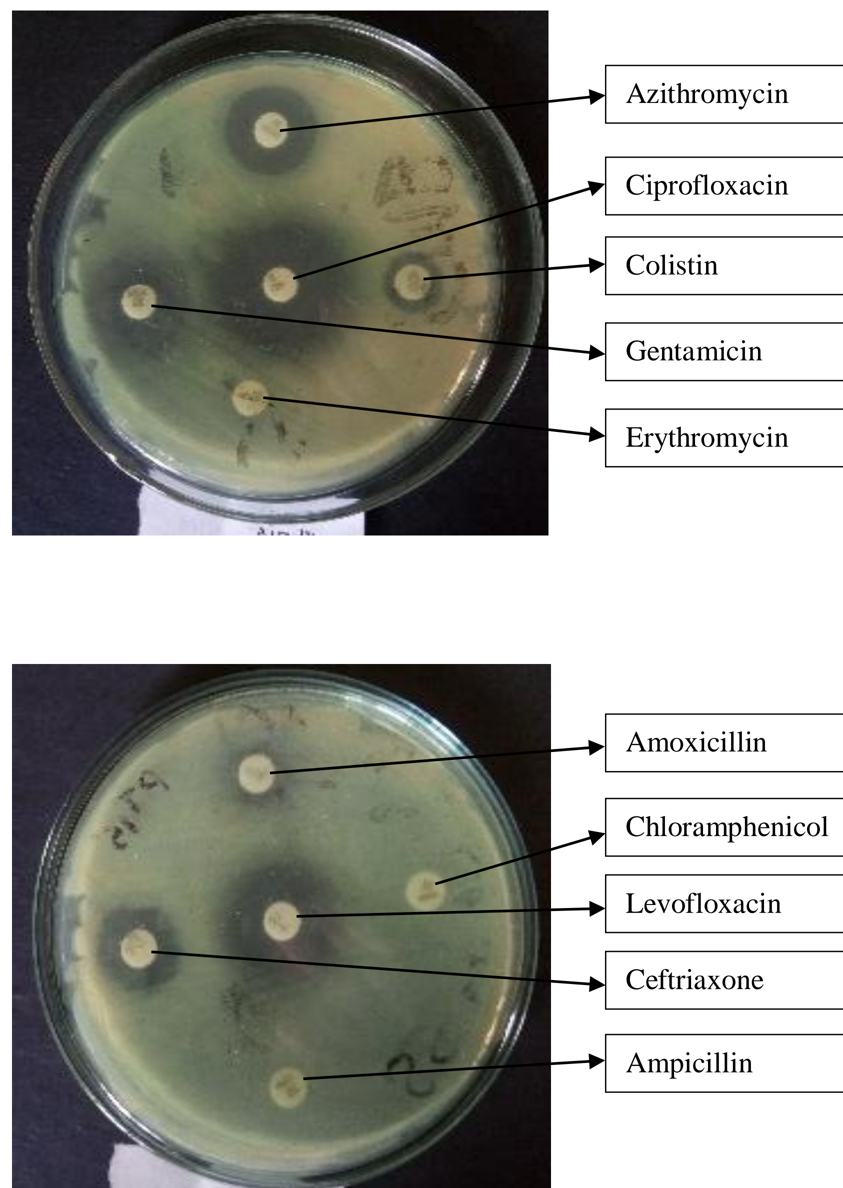


Fig. 4.19: Antibiotic sensitivity test of *Vibrio* spp.

Table 4.30: Results of Antimicrobial susceptibility of the isolated *Vibrio* spp from tap water.

Name of isolates	No. (%)									
E. coli (n=15)	GEN	CIP	C	LE	CTR	CL	AZM	AMP	AMX	E
Susceptible	10(66.66)	10(66.66)	2(13.33)	8(53.33)	11(73.33)	6(40)	0	0	0	1(6.66)
Intermediate	3(20)	3(20)	4(26.66)	5(33.33)	4(26.66)	9(60)	7(46.66)	5(33.33)	5(33.33)	5(33.33)
Resistant	2(13.33)	2(13.33)	9(60)	2(13.33)	0	0	8(53.33)	10(66,66)	10(66,66)	9(60)

Note: GEN=Gentamycin, CIP =Ciprofloxacin, C=Chloramphenicol, LE=Levofloxacin, CTR= Ceftriaxone, CL=Colistin, AZM= Azithromycin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin

I=Intermediate, S=Susceptible and R=Resistance

4.10 Polymerase Chain Reaction (PCR)

DNA amplification, sequencing of 16 rRNA genes with universal primers and analysis of *E. coli* and *vibrio* spp.

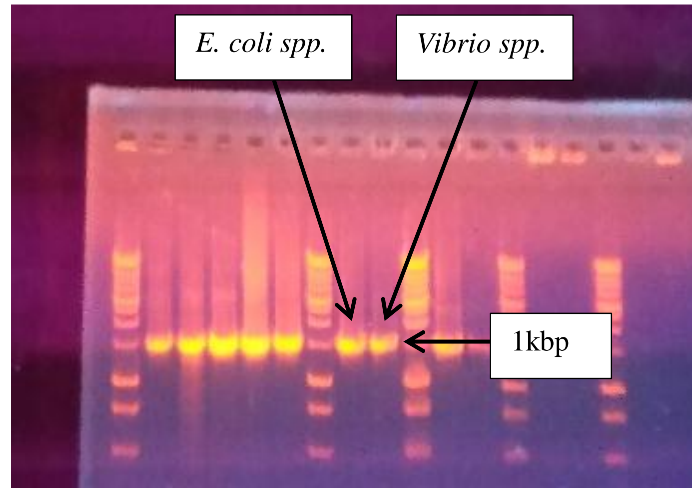


Fig. 4.20: Result of PCR targeting 16S rRNA gene for the identification of *E. coli and Vibrio* spp

CHAPTER 5

DISCUSSION

This paper investigates the association between water quality and selected management practices by users among the human dairy farm and poultry farm in dinajpur district of Bangladesh. Total ninety (90) samples, thirty from human tube-well water, thirty water samples of animal farm and thirty water samples of poultry farm were collected and tested for TVC (Total Viable Count) and MPN (Most Probable Number) to assess the general microbiological quality of drinking water.

The geometric mean of HPC (Heterotrophic Plate Count) of tube-well water were in Sadar 2.55×10^3 CFU mL^{-1}, Birgonj 3.11×10^3 CFU mL^{-1}, Kaharol 2.85×10^3 CFU mL^{-1}, Birol 2.88×10^3 CFU mL^{-1} and Chirirbandar 3.20×10^3 CFU mL^{-1}.The highest HPC was found in Chirirbandar upazilla 3.20×10^3 CFU mL^{-1} and lowest HPC was found in Sadar upazilla 2.55×10^3 CFU $mL^{-1.}$ On the other hand, the MPN values 2.16, 2.16, 2.50, 1.83 and 2.33 coliforms/100 ml were found in Sadar, Birgonj, Kaharol, Birol and Chirirbandar upazilla respectively. The highest MPN value was found in Kaharol 2.50 coliforms/100 ml and lowest were found in sadar 2.16 coliforms/100 ml and Chirirbandar 2.16 coliforms/100 ml .This result is similar to (**Kabir *et al.,* 2015**).Total bacterial count and total coliform count of tubewell drinking water for human consumption has a acceptable limit. In most cases, the pipe system is very old and most of the pipes are poor in condition. There are leakage and breakage through which contaminants from outside the pipe might enter and get mixed with the tubewell water. Both of these phenomena might cause easier entrance of contaminants into pipe lines. All taken together, the overall picture showed that the underground water sources are not free from bacterial contamination.

An adequate supply of good quality water for dairy farm is extremely important for optimal production.The geometric mean of HPC of animal farm tubewell water were Sadar 2.3×10^3 CFU mL^{-1}, Birgonj 3.6×10^3 CFU mL^{-1}, Kaharol 2.7×10 CFU ^{3} mL^{-1}, Birol 3.2×10^3 CFU mL^{-1} and Chirirbandar 3.4×10^3 CFU mL^{-1}. The highest HPC was found in Birgonj 3.6×10^3 CFU mL^{-1} and lowest HPC was found in Sadar 2.3×10^3 CFU mL^{-1}. On the other hand, the MPN values were 1.66, 2.33, 2.66,2.00 and 2.66 coliforms/100 ml in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. In this study, it was found that the highest MPN values were found in Kaharol 2.66 coliforms/100 ml and

Chirirbandar 2.66 coliforms /100 ml and lowest was found in sadar 1.66 coliforms/100 ml of water.

The geometric mean of HPC of dairy farm manger water were Sadar 4.66×10^7 CFU mL^{-1}, Birgonj 2.0×10^7 CFU mL^{-1},Kaharol 2.8×10^7 CFU mL^{-1}, Birol 2.8×10^7 CFU mL^{-1} and Chirirbandar 4.2×10^7 CFU mL^{-1}. In this study, it was found that the highest geometric mean of HPC of animal dairy manger water were in Sadar 4.66×10^7 CFU mL^{-1} and lowest HPC was found in Birgonj 2.0×10^7 CFU mL^{-1}. On the other hand, the MPN values were 14.00,17.33,14.00,16.33 and 15.33 coliforms /100 ml Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. In this study, it was found that MPN of dairy farm manger water was highest in Birgonj 17.33 coliforms /100 ml and lowest were found in Sadar 14.00 coliforms /100 ml and Kaharol 14.00 coliforms /100 ml. Total bacterial count and total coliform count of tubewell and manger drinking water for the uses of dairy farm have a acceptable limit.The extent of bacterial contamination observed in the drinking water troughs but not in initial source tubewell may reveal that animal get exposure to bacterial infection from drinking water because troughs are exposed to contamination from many sources like cattle itself while drinking, animal faeces, air, dust, feed stuffs, farm stuffs and improper cleaning of troughs.The findings of the present study correlated with the findings of **Musa *et al.,* (2014)** which values are slighthlly higher.

The geometric mean of HPC of Poultry farm tubewell water were Sadar 2.8×10^3 CFU mL^{-1}, Birgonj 2.67×10^3 CFU mL^{-1}, Kaharol 3.0×10^3 CFU mL^{-1}, Birol 2.57×10^3 CFU mL^{-1} and Chirirbandar 3.0×10^3 CFU mL^{-1}.In this study, it was found that HPC of Poultry farm tubewell water was highest in Kaharol 3.0×10^3 CFU mL^{-1} and lowest in Birol 2.57×10^3 CFU mL^{-1}.On the other hand, the MPN values of Poultry farm tubewell water were 2.66, 2.00, 2.33, 1.66 and 2.00 coliforms/100 ml of drinking water in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. In this study, it was found that MPN of Poultry farm tubewell water was highest in Sadar 2.66 coliforms/100 ml and lowest in Birol 1.66 coliforms/100 ml.

The geometric mean of HPC of Poultry farm waterer water Sadar 4.2×10^7 CFU mL^{-1},Birgonj 2.00×10^7 CFU mL^{-1}, Kaharol 3.25×10^7 CFU mL^{-1},Birol 1.00×10^7 CFU mL^{-1}, and Chirirbandar 2.00×10^7 CFU mL^{-1}. In this study, it was found that HPC of Poultry farm waterer water was highest in Sadar 4.2×10^7 CFU mL^{-1} and lowest in Birol 1.00×10^7

CFU mL^{-1}.On the other hand, the MPN values were 12.33, 9.66, 10.33, 13.33 and 12.33 coliforms/100 ml waterer water found in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively.In this study, it was found that the highest MPN value poultry farm waterer was found in Birol 13.33 coliforms/100 ml and lowest were found in Birol 9.66 coliforms/100 ml. Total bacterial count and total coliform count of tubewell and waterer drinking water for the uses of poultry farm have a acceptable limit.The persistently high value of bacterial load observed in the waterer samples than original tubewell water, the possibility of high level of contamination by the birds, waterer being soiled with their digesta could also be contaminated with their feaces and even when the birds dust-bath, litter materials could enter and further contaminate the water troughs. When the water troughs are not properly washed, could create favourable environment for the growth and proliferation of bacterial organisms when subsequently refilled without cleaning The findings of the present study correlate with the findings of **Folorunso *et al.*, (2014)** which are higher values.

Cultural *techniques, Grams staining techniques and* biochemical test were applied for isolation and identification of bacterial pathogen from ninety (90) water samples.Seven bacteria were isolated such as *Escherichia coli, Klebsiella* spp, *Salmonella* spp, *Shigella* spp, *Vibrio* spp, *Staphylococcus* spp and *Pseudomonas* spp, among them the most frequent was *Escherichia coli* 82(91.11%) followed by *Salmonella* spp 81(90.00%), *Shigella* spp 80(88.90%), *Klebsiella* spp 79(87.77%), *Vibrio* spp 73(81.11%), *Pseudomonas* spp 60(66.66%) and *Staphylococcus* spp 59(65.55%). The antimicrobial susceptibility pattern showed that the isolates were highly resistant to ampicillin, amoxicillin, erythromycin and chloramphenicol and susceptible to gentamicin, azithromycin, colistin, ceftriaxone, levofloxacin and ciprofloxacin. This study indicated the presence of multidrug resistant *bacteria* isolates in tubewell water in Dinajpur districts that warrant particular attention.This might be due to haphazard use of antibiotic without following rules and regulation of antibiotic use in both human, animal and poltry health and or use meat of animal and poultry without maintain appropriate antibiotic residual withdrawal period.Out of seven bacteria two bacteria *(Escherichia coli* and *Vibrio* spp) are subjected to amplified by using 16S rRNA gene based PCR.

CHAPTER 6
SUMMARY AND CONCLUSSION

The geometric mean of Heterotrophic Plate Count of tubewell water of human were found in Sadar 2.55×10^3 CFU mL^{-1}, Birgonj 3.11×10^3 CFU mL^{-1}, Kaharol 2.85×10^3 CFU mL^{-1}, Birol 2.88×10^3 CFU mL^{-1} and Chirirbandar 3.20×10^3 CFU mL^{-1}.The highest HPC was found in Chirirbandar upazilla 3.20×10^3 CFU mL^{-1} and lowest HPC was found in Sadar upazilla 2.55×10^3 CFU $mL^{-1.}$ On the other hand, the MPN values 2.16, 2.16, 2.50, 1.83 and 2.33 coliforms/100 ml were found in Sadar, Birgonj, Kaharol, Birol and Chirirbandar upazilla respectively. The highest MPN value was found in Kaharol 2.50 coliforms/100 ml and lowest were found in sadar 2.16 coliforms/100 ml and Chirirbandar 2.16 coliforms/100 ml. Tubewell must be established in dry and high land where chance of water accumulation is low and subsequent lower chance of environment pollution. The pipe system condition must be improve to prevent leakage and breakage of pipe and prohibit contmination of tubewell water with sweage disposal. Both of these phenomena might cause easier entrance of contaminants into pipe lines. All taken together, the overall picture showed that the underground water sources are not free from bacterial contamination. A proper sanitation and drainage network system in the city and rural area is a priority now a day.

The geometric mean of HPC of dairy farm tubewel water were Sadar 2.3×10^3 CFU mL^{-1}, Birgonj 3.6×10^3 CFU mL^{-1}, Kaharol 2.7×10 CFU ^{3} mL^{-1}, Birol 3.2×10^3 CFU mL^{-1} and Chirirbandar 3.4×10^3 CFU mL^{-1}. The highest HPC was found in Birgonj 3.6×10^3 CFU mL^{-1} and lowest HPC was found in Sadar 2.3×10^3 CFU mL^{-1}. On the other hand, the MPN values were 1.66, 2.33, 2.66,2.00 and 2.66 coliforms/100 ml in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. In this study, it was found that the highest MPN values were found in Kaharol 2.66 coliforms/100 ml and Chirirbandar 2.66 coliforms /100 ml and lowest was found in sadar 1.66 coliforms/100 ml of water.

The geometric mean of HPC of dairy farm manger water were Sadar 4.66×10^7 CFU mL^{-1}, Birgonj 2.0×10^7 CFU mL^{-1},Kaharol 2.8×10^7 CFU mL^{-1}, Birol 2.8×10^7 CFU mL^{-1} and Chirirbandar 4.2×10^7 CFU mL^{-1}. In this study, it was found that the highest geometric mean of HPC of dairy farm manger water were in Sadar 4.66×10^7 CFU mL^{-1} and lowest HPC was found in Birgonj 2.0×10^7 CFU mL^{-1}. On the other hand, the MPN values were 14.00,17.33,14.00,16.33 and 15.33 coliforms /100 ml Sadar, Birgonj, Kaharol, Birol and

Chirirbandar respectively. In this study, it was found that MPN of dairy farm manger water was highest in Birgonj 17.33 coliforms /100 ml and lowest were found in Sadar 14.00 coliforms /100 ml and Kaharol 14.00 coliforms /100 ml. To avoid bacterial contamination of manger water manger must be properly clean with disinfectant at regular basis. Animal handler must be remain proper clean,diseased free and maintain own hygiene before and after handling, feeding and watering of animal. Diseased animal must be isolated and treated appropriately because animal sometimes act as a source of infection while it is drinking together with healthy animal. Proper management of cowdung is also essential because cowdung when get mixed with drinking water it become unhygienic and not suitable for consumption and may affect health hazard of animal.

The mean of HPC of Poultry farm tubewell water were Sadar 2.8×10^{3} CFU mL^{-1}, Birgonj 2.67×10^{3} CFU mL^{-1}, Kaharol 3.0×10^{3} CFU mL^{-1}, Birol 2.57×10^{3} CFU mL^{-1} and Chirirbandar 3.0×10^{3} CFU mL^{-1}.In this study, it was found that HPC of Poultry farm tubewell water was highest in Kaharol 3.0×10^{3} CFU mL^{-1} and lowest in Birol 2.57×10^{3} CFU mL^{-1}.On the other hand, the MPN values of Poultry farm tubewell water were 2.66, 2.00, 2.33, 1.66 and 2.00 coliforms/100 ml of drinking water in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively. In this study, it was found that MPN of Poultry farm tubewell water was highest in Sadar 2.66 coliforms/100 ml and lowest in Birol 1.66 coliforms/100 ml.

The geometric mean of HPC of Poultry farm waterer water Sadar 4.2×10^{7} CFU mL^{-1}, Birgonj 2.00×10^{7} CFU mL^{-1}, Kaharol 3.25×10^{7} CFU mL^{-1},Birol 1.00×10^{7} CFU mL^{-1}, and Chirirbandar 2.00×10^{7} CFU mL^{-1}. In this study, it was found that HPC of Poultry farm tubewell water was highest in Sadar 4.2×10^{7} CFU mL^{-1} and lowest in Birol 1.00×10^{7} CFU mL^{-1}.On the other hand, the MPN values were 12.33, 9.66, 10.33, 13.33 and 12.33 coliforms/100 ml waterer water found in Sadar, Birgonj, Kaharol, Birol and Chirirbandar respectively.In this study, it was found that the highest MPN value was found in Birol 13.33 coliforms/100 ml and lowest were found in Birol 9.66 coliforms/100 ml.The persistently high value of bacterial load observed in the waterer samples than original tubewell water, the possibility of high level of contamination by the birds, waterer being soiled with their digesta could also be contaminated with their feaces and even when the birds dust-bath, litter materials could enter and further contaminate the water troughs.

The water troughs should be properly washed on regular basis to prevent favourable environment for the growth and proliferation of bacterial organisms .

Cultural techniques, Grams staining techniques and biochemical test were applied for isolation and identification of bacterial pathogen from ninety water samples.Seven bacteria were isolated such as *Escherichia coli, Klebsiella* spp, *Salmonella* spp, *Shigella* spp, *Vibrio* spp, *Staphylococcus* spp and *Pseudomonas* spp*,* among them the most frequent was *Escherichia coli* 82(91.11%) followed by *Salmonella* spp 81(90.00%), *Shigella* spp 80(88.90%), *Klebsiella* spp 79(87.77%), *Vibrio* spp 73(81.11%), *Pseudomonas* spp 60(66.66%) and *Staphylococcus* spp 59(65.55%). The antimicrobial susceptibility pattern showed that the isolates were highly resistant to ampicillin, amoxicillin, erythromycin and chloramphenicol and susceptible to gentamicin, azithromycin, colistin, ceftriaxone, levofloxacin and ciprofloxacin. Haphazard use of antibiotic must be prohibited, must be follow rules and regulation of antibiotic uses in both human, dairy and poultry health and or use meat of cattle, poultry and dairy products after maintainage of appropriate antibiotic residual withdrawal period.Some antibiotic which are use as a reserve drugs must not use indiscriminately. Out of seven bacteria two bacteria *(Escherichia coli* and *Vibrio* spp) are subjected to amplified by using 16S rRNA gene based PCR. Therefore, regular monitoring and assessment of drinking water is primarily a health-based activity which helps to protect public health through ensuring provision of quality water. Bad habits, water mishandling and lack of basic knowledge affects clearly the quality of water in animal and poultry farms; thus physical appearance of water had been clearly affected, and this could strongly result in bad hygiene situation, causing a high level in the incidence of water-borne disease.

RECOMMENDATIONS

1. The possible consequence of unhygienic drinking water consumption is fatal so proper management of water is very important and should never be compromised.
2. Water analysis for the detection of faecal pollution should be prompted to determine the level of faecal pollution in ground water resources whenever water is intended for animal and human use.

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APPENDICES

APPENDIX 1

Composition of Different Media

1. Nutrient agar (Hi Media)

Ingredients:	g/L
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 250C)	7.4 ± 0.2

2. Eosine methylene blue Agar (Hi Media)

Ingredients:	g/L
Peptic digest of animal tissue	10
Lactose	5.0
Sucrose	5.0
Dipotassium phosphate	2.0
Eosin - Y	0.40
Methylene blue	0.065
Agar	20.0
Final pH (at 250C)	7.2 ± 0.2

3. MacConkey agar (Hi-media) Ingredients:	**g/L**
Peptic digest of animal tissue	17.0
Protease peptone	3.0
Lactose monohydrate	10
Bile salt	1.5
Sodium chloride	5.0
Agar-agar	15.0
Neutral red	0.03
Final pH (at 250C)	7.1 ± 0.2

4. Simmon's Citrate Agar

Component	Amount (g/L)
Magnesium sulphate	0.2
Ammoniun dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bacto agar	15.0
Bacto bromo thymol blue	0.08

5. Mueller Hinton Agar

Component	Amount (g/L)
Beef infusion	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH(at 25°C)	7.3±0.1

6. TSI agar (Hi Media)

Ingredients:	g/L
Peptic digest of animal tissue	10.00
Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Beef extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.00
Final pH(at 25°C)	7.4 ± 0.2

7. MIU medium base (Hi Media)

Ingredients:	g/L
Casein enzymic hydrolysate	10.00
Dextrose	1.00
Sodium chloride	5.00
Phenol Red	0.01
Agar	2.00
Final pH(at 25°C)	6.8 ± 0.2

8. MR-VP medium (Hi Media)

Ingredients:	g/L
Buffered peptone	7.00
Dextrose	5.00
Dipotassium phosphate	5.00
Final pH (at 25°C)	6.9 ± 0.2

APPENDIX 2

Preparation of reagents

1. Kovacs reagent

P-dimethyl aminobenzal dehyde	5 gm
Amylalcoho	175 gm
Conc. HCL	25 ml

2. V-P reagent 1

5% alpha –naptholin absolute ethyl alcohol

3. V-P reagent 2

40% potassium hydroxide containing 0.3creatine. The ingredients were dissolved by heating gently over steam bath. When in solution add 0.05gm of cotton blue dye.

4. Phosphate buffered solution

Sodium chloride	8 gm
Disodium hydrogen phosphate	2.8 gm
Potassium chloride	0.2 gm
Potassium hydrogen phosphate	0.2 gm
Distilled water to make	1000 ml

5. Methyl red solution

Methyl red	0.05 gm
Ethanol (absolute)	28 ml
Distilled water	22 ml

6. Phenol red solution

0.2% aqueous solution of phenol red

7. Potassium hydroxide solution

40% aqueous solution of KOH

8. Gram stain solution

Stock crystal violet

Crystal violet	10 gm
Ethyl alcohol (95%)	1000 ml

Stock oxalate solution

Ammonium oxalate	1 gm
Distilled water	1000 ml

Lugols iodine solution

Iodine crystal	1 gm
Potassium iodide	2 gm
Ethyl alcohol	250 m
Acetone	250 m

Counterstain

Safranine	2.5 ml
Ethyl alcohol (95%)	100 ml

Safranine working solution

The stock safranine is diluted 1:4 with distilled water.