ISOLATION, IDENTIFICATION AND ANTIBIOGRAM STUDY OF BACTERIAL PATHOGENS FROM STORED WATER IN TANKS AT HSTU MALE HALLS

A THESIS

BY

KHADAR GOOD NOUR

REGISTRATION NO. 1805500

SEMESTER: JULY-DECEMBER, 2019

SESSION: 2018

MASTER OF SCIENCE (M.S.)
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Submitted to the

Department of Microbiology

Hajee Mohammad Danesh Science and Technology University, Dinajpur

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (M.S.)

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DECEMBER, 2019

Dedicated

To

My Beloved Sister

ACKNOWLEDGEMENTS

All praises are to the almighty Allah, who enables the author to complete the research work successfully and to submit the thesis leading to degree of Master of Science (MS) in Microbiology., The author would like to express his deepest sense of gratitude and indebtedness to his honorable teacher and research Supervisor, Professor Dr. Md. Mostafizer Rahman Ph.D., Chairman, Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh for his invaluable and critical suggestions, scientific acumen perspicacious remarks, scholarly guidance, active persuasion and supervision, which served as a constant source of inspiration throughout the course of my study and research work.

The author finds it a great pleasure in expressing his heartful gratitude and immense indebtedness to my honorable, respected teacher and co-supervisor, Dr. Mir Rowshan Akter, Professor Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her scholastic supervision, kind co-operation, inspiration, to conduct the course work and research smoothly and perfectly.

The author humbly desires to express his profound respect and sincere appreciation to his respective teachers Professor Dr. Md. Khaled Hossain, Dr. Farzana Afroz, Assistant Professor, Dr. Nazmi Ara Rumi, Assistant Professor & Dr. Mst. Deloara Begum, Assistant Professor Department of Microbiology, HSTU, Dinajpur, for their encouragement, valuable suggestions and kind co-operation throughout the course of the study and research smoothly and perfectly.

The author is grateful to his beloved parents mother Asha Colad Jama and my father Good Nour for their sacrifices, blessing, moral support and encouragement from the beginning of his study period in HSTU, Dinajpur.

With due pleasure the author wishes to acknowledge the healthy working relationship of laboratory technicians and office staffs Md. Ayub Hossain Md. Ansar Ali and Md. Saiful Islam, department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

The author is grateful to his laboratory cheif in Hargiesa group Hospital Ahmed hashi Farah for his moral support The author is grateful to his friends for their cordial help and suggestion in the preparation of the manuscript. Finally, the author would like to express grate full thanks to his all well-wishers.

The author December, 2019

ABSTRACT

The present study was conducted to isolate, identify and Antibiogram study bacteria from stored water in tanks of some selected Tanks at HSTU Male Halls in Dinajpur district of Bangladesh. total of 36 samples were collected Aseptically from different Tanks, During this period from August to November 2019. The stored water are collected and brought to the laboratory under the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur the stored water different bacteriological tests for isolation allowed identification of bacteria based on morphology, staining, cultural and biochemical examinations and Antibiogram study. The study revealed that the total prevalence of bacterial pathogens isolated and identified from stored water samples were *E. coli* 11(30%) Salmonella spp 8(22%) *Klebsiella* spp 2(5.5% *Shigella* spp. 5(13.9%) Staphylococcus spp. 5(13.9%) and Pseudomonas spp. 2(5.5%) *Vibrio spp* (2.8%) *Micrococcus* 2 (5.5%) The Most effective antibiotics against *Salmonella* spp, Shigella spp, Pseudomonas spp, staphylococcus spp, *Vibrio spp,* micrococcus spp is Streptomycin, Chloramphenicol, Amikacin, moxifloxacin were Ampicilin, Amoxycillin and Tetracycline are Resistant In the context of this study, it may be concluded that: The presence of *E. coli*, Salmonella spp. Klebsiella spp., Shigella spp., Staphylococcus and Pseudomonas presents stored water in Tanks Wich Makes health hazards to both students and Other staffs to cause virous Disease such as diarrhea, dysentery, cholera, typhoid and gastroenteritis. The antibiotic resistance properties of isolated bacteria can cause serious health hazards because of ineffective treatment of the sufferer's by the commonly described antibiotics.

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LIST OF ABBREVIATIONS

- : Negative

% : Percentage

+ : Positive

℃ : Degree of Celsius

µg : Microgram

Assist : Assistant

CEF : Cefixime

CIP : Ciprofloxacin

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 H2O2

H_2O_2 : Hydrogen peroxide

H_2S : Hydrogen sulphide

HPC : Heterotrophic Plate Count

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

MPN : Most Probable Number

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

TVC : Total Viable Count

v/v : Volume by volume

VP : Voges-Proskauer

CHAPTER 1

INTRODUCTION

Water is essential for all living beings. Without water, life can not be sustained beyond a few days. 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 **(**Geldreich, 1990).

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 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 (Jackson *et al.*, 2001).

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 (Michael *et al.*, 2006).

A large number of infectious diseases are spread primarily through water supplies contaminated with human and animal excreta predominantly faeces (Hannan *et al.,* 2010).

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 less than 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 a 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).

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 Important factors to prevent waterborne diseases in water stored tanks production are the protection of supply sources, water disinfection and the quality control of

microbiological, chemical and physical characteristics. In many counters water stored tanks owner have little knowledge of the effect of poor tank hygiene practices resulting outbreak of infectious diseases occur in the in people **(**Jackson *et al.,* 2001).

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 Microbiological examination of water should routinely be carried out to control the quality of drinking water 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 **(**Ferretti *et al.,* 2010).

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.

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

Objectives

- To determine the bacterial load in Stored water of Different Tanks in HSTU Male
- To isolation and identification of bacteria from collected samples
- Antibiogram study

CHAPTER 2

REVIEW OF LITERATURE

2.1 To determine the bacterial load in Stored water of Different Tanks in HSTU Male Halls

Luby *et al.*, (2015**)** evaluated to assess the relationship between drinking water microbiological quality and child diarrhea. We included 50 villages across rural Bangladesh. Within each village field-workers enrolled a systematic random sample of 10 households with a child under the age of 3 years. Community monitors visited households monthly and recorded whether children under the age of 5 years had diarrhea in the preceding 2 days. Every 3 months, a research assistant visited the household and requested a water sample from the source or container used to provide drinking water to the child. 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 log 10 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.

Khan *et al.* (2013) described Access to safe drinking water is one of the basic human rights and essential for healthy life. The present study 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 for physical parameters, anions, heavy metals and coliform bacteria using standard methods. The concentrations of nitrate (10.3–14.84 mg L^{-1}) in 13 sites exceeded the permissible limit (10 mg L^{-1}) set by US-EPA, while sulfate concentrations (505–555 mg L^{-1}) in 9 sites exceeded the permissible limit (500 mg L^{-1}) set by WHO. Similarly, the concentrations of Pb, Cd, Ni and Fe exceeded their respective permissible limits set by different organizations in some locations. Furthermore, 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. In the study area, improper

disposal of sewage and solid wastes, over application of agrochemicals (pesticides and fertilizers), deteriorating condition of piping network and transportation were the major sources responsible for 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. In order to reduce the health risk, it is necessary to immediately stop the uses of drinking water from contaminated sources and government should supply treated/clean water with supply lines far away from solid waste, sludge and sewage sites. The farmers should be properly trained to avoid the overusing of agrochemicals responsible for drinking water contamination, while both women and men should be properly educated with water knowledge through awareness and training programs needed for sustainable use and management of drinking water.

Levantesi *et al.,* (2012**)** described that salmonella is one of the main causes of intestinal disorder all over the world as well as the causal agent of more severe systemic diseases such as typhoid and paratyphoid fevers. While water is known to be a most common vehicle for the transmission of Typhoid Salmonella serovars, non-typhoidal salmonellae are mainly known as food borne pathogens. This paper provides a brief review of the last ten years of peer reviewed publications on the prevalence of Salmonella in natural freshwaters and drinking waters,

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) All the samples were found contaminated with total coliform bacteria and unfit for human consumption according to WHO standards.

Barati, (2011) Identified Generally, drinking water contains two categories of microorganisms first is known as persistent microorganisms which naturally settle in water, with little food needs, and it includes Acinetobacter, Flavobacerium and Chromobacterium species while other group is transient microorganisms which are

transmitted to water from the environment, soil, human or animal, and pathogens fall into this category.

Girones *et al.,* (2010**)** Identified Pollution of water by sewage and run-off from farms produces a serious public health problem in many countries. Viruses, along with bacteria and protozoa in the intestine or in urine are shed and transported through the sewer system. Even in highly industrialized countries, pathogens, including viruses, are prevalent throughout the environment. Molecular methods are used to monitor viral, bacterial, and protozoan pathogens, and to track pathogen- and source-specific markers in the environment. Molecular techniques, specifically polymerase chain reaction-based methods, provide sensitive, rapid, and quantitative analytical tools with which to study such pathogens, including new re-emerging strains. These techniques are used to evaluate the microbiological quality of food and water, and to assess the efficiency of virus removal in drinking and waste water treatment plants. The range of methods available for the application of molecular techniques has increased, and the costs involved have fallen. These developments have allowed the potential standardization and automation of certain techniques.In some cases they facilitate the identification, genotyping, enumeration, viability assessment, and source-tracking of human and animal contamination. Additionally, recent improvements in detection technologies have allowed the simultaneous detection of multiple. ‘targets in a single assay. However, the molecular techniques available today and those under development require further refinement in order to be standardized and applicable to a diversity of matrices. Water disinfection treatments may have an effect on the viability of pathogens and the numbers obtained by molecular techniques may overestimate the quantification of infectious microorganisms. The pros and cons of molecular techniques for the detection and quantification of pathogens in water are discussed.

Taulo *et al.* (2008**)** investigated and compared the microbiological quality of source, transported and stored water in Lungwena households. It also 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) Identified *Escherichia coli* is naturally present in the intestinal tracts of warm-blooded animals. Since *E. coli* is released into the environment through deposition of fecal material, this bacterium is widely used as an indicator of fecal contamination of waterways. Recently, research efforts have been directed towards the identification of potential sources of fecal contamination impacting waterways and beaches. This is often referred to as microbial source tracking. However, recent studies have reported 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.

Nathaniel *et al.,* (2008) Examined from over 100 sources in Nepal's Kathmandu Valley, including municipal taps, dug wells, shallow-aquifer tube wells, deep-aquifer tube wells, and dhungedharas (or stone spouts, public water sources that capture groundwater or surface water). Information was gathered on user preference and site and well characteristics, and water was examined for indicators of contamination from sewage, agriculture, or industry. Most problematic were total coliform and Escherichia coli bacteria, which were present in 94 and 72% of all the water samples, respectively. Contamination by nitrate, ammonia and heavy metals was more limited; nitrate and ammonia exceeded Nepali guidelines in 11 and 45% of the samples, respectively. Arsenic and mercury exceeded WHO guidelines in 7 and 10% of the samples, respectively, but arsenic never exceeded the less strict Nepali guideline. Significant

differences existed in contamination levels between types of sources; dug wells and dhungedharas, 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.

September *et al.*, (2007) investigated Water for human consumption is required to be free from any bacteria that might pose a health risk. The presence of biofilms in the drinking water distribution system may play a role in the presence of potential pathogens in the drinking water supply. 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 units 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.

Mario *et al.*, (2005) isolated the notorious pathogens *Mycobacterium tuberculosis* and *M. leprae*, the majority of the mycobacterium species described to date are generally not considered as obligate human pathogens. The natural reservoirs of these non-primary pathogenic mycobacterium include aquatic and terrestrial environments. Under certain circumstances, e.g., skin lesions, pulmonary or immune dysfunctions and chronic diseases, these environmental mycobacterium (EM) may cause disease. EM such as *M. avium, M. kansasii*, and *M. xenopi*have frequently been isolated from drinking water and hospital water distribution systems. Biofilm formation, amoeba-associated lifestyle, and resistance to chlorine have been recognized as important factors that contribute to the survival, colonization and persistence of EM in water distribution systems.

Schets *et al.*,(2005) Observed The microbiological quality of drinking water from 144 private water supplies in the Netherlands was tested and additionally the occurrence of *Escherichia coli* O157 was examined. Faecal indicators were enumerated by using standard membrane filtration methods. The presence of *E. coli*O157 was determined 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. Pulsed field gel electrophoresis (PFGE) analysis suggested that cattle might have been the cause of contamination.

Our results indicate that compliance with microbiological quality standards obtained in routine monitoring does not always guarantee the absence of pathogens. The presence of pathogens such as *E. coli* O157 may suggest possible health consequences; however, a risk assessment process should be performed as the monitoring of both faecal indicator parameters and pathogens do not predict the effect of microbial contamination of drinking water on a population.

Ashbolt *et al.,* (2004) observed Drinking water is a major source of microbial pathogens in developing regions, although poor sanitation and food sources are integral to enteric pathogen exposure. Gastrointestinal disease outcomes are also more severe, due to under-nutrition and lack of intervention strategies in these regions. Poor water quality, sanitation and hygiene account for some 1.7 million deaths a year world-wide (3.1% of all deaths and 3.7% of all DALY's), mainly through infectious diarrhea. Nine out of 10 such deaths are in children and virtually all of the deaths are in developing countries. Major enteric pathogens in these children include: rotavirus, *Campylobacter jejuni*, enter toxigenic *Escherichia coli*, *Shigella* spp. and *Vibrio cholerae* O1, and possibly enteropathogenic *E.coli*, *Aeromonas* spp. *V. cholerae* O139, enterotoxigenic *Bacteroides fragilis*, *Clostridium difficile* and *Cryptosporidium parvum*. All except the latter are easily control by chlorination of water, but recontamination of treated water is a huge problem. Emerging environmental pathogens, such as *Helicobacter pylori* and *Burkholderiapseudomallei*, may well be of significance in some regions. In adults, much less is understood of various sequellae such as myocarditis, diabetes, reactive arthritis and cancers some months–years after initial infections. So in addition to the traditional

pathogens (helminths, *Entamoebahistolytica*, *Giardia lamblia*hepatitis A and E) various enteroviruses, *C. jejuni*and*H. pylori* are emerging issues.

Asano *et al.*, (2004) Evaluated Groundwater recharge 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 waste water. At present, some uncertainties with respect to health risk insiderations 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. In addition, a summary is provided of the methodology used in developing the World Health Organization's Guidelines for drinking Water Quality to illustrate how numerical guideline values are generated for contaminants that may be applicable to ground water recharge.

Toze *et al.*, (1999) Observed Microbial pathogens are one of the major health risks associated with water and waste waters Current methods for the detection of pathogenic viruses, bacteria, protozoa and helminthes tend to be inaccurate, time consuming and expensive As a result, indicator bacteria are commonly used to determine 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.

2.2 To isolation and identification of bacteria from collected samples

Farzana *et al.*, (2014) Identified More than 20,000 children die annually in the city of Karachi alone, majority of whose death are thought to be associated with waterborne pathogens. Drinking water and recreational exposure to polluted water pose a significant public health threat including gastroenteritis, paralysis, meningitis, hepatitis, respiratory illness and diarrhea. The aim of this study was to determine the presence of bacterial contamination in drinking water supplies in Karachi, Pakistan. A total of fifty two

domestic tap water samples were collected 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 quality

Prasai *et al.,* (2011) observed Drinking water quality assessment in Kathmandu valley has always been crucial with reference to public health importance. A study was conducted to evaluate the quality of drinking water of the valley. 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 *Salmonellatyphi*, 3.0% were Proteus vulgaris, 3.0% were *Serratia*spp and 1.0% were Vibrio cholera.

Figueras *et al.,* (2010) evaluated The safety of drinking water is by the results obtained from faecal indicators during the stipulated controls fixed by the legislation. However, drinking-water related illness outbreaks are still occurring worldwide. The failures that lead to these outbreaks are relatively common and typically involve preceding heavy rain and inadequate disinfection processes. The role that classical faecal indicators have played in the protection of public health is reviewed and the turning points expected for the future explored. The legislation for protecting the quality of drinking water in Europe is under revision, and the planned modifications include an update of current indicators and methods as well as the introduction of Water Safety Plans (WSPs), in line with WHO recommendations. The principles of the WSP approach and the advances signified

by the introduction of these preventive measures in the future improvement of drinking water quality are presented. The expected impact that climate change will have in the quality of drinking water is also critically evaluated.

Bassam *et al.,* (2008**)** Examined Eighty six samples of Sabil water were collected during 1929 of Ramadan 1428A.H. (1-10 October 2007) and examined using standard methods of analyses for drinking water, which include viable counts for total viable bacterial count (TVB), coliform bacteria count and presence of *E. coli*. In addition, water samples were examined for the presence of potential pathogens, and tested for their resistance to antibiotics by the disc diffusion method. Also, the occurrence of filamentous fungi together with bacteriological parameters was assessed in this study. 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*, *Aeromonasveronii*, *Acinetobacter* spp. and Aeromonashydrophila. While the most frequently isolated fungal species were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Epidermophtton* spp., *Microsporum* spp., *Penicillium* spp. The high bacterial contamination in Sabil water is related to human skin transmission and, probably to misuse of the water, such as using the same cups for drinking to perform the ritual washing, i.e. wodow (ablution).

2.3 Anti biogram Study

Sanganyado *et a.,*. (2019**)** Determined in recent years, there has been a growing interest on the occurrence of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in treated and untreated drinking water. 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 envir onmental 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. The dose-response

relationships of pathogenic ARB in drinking water, which is required for accurate risk assessments.

Romya *et al.*, (2013) described that sensitivity of *Salmonella Enteritidis* was 100% for Ciprofloxacin followed by Chloramphenicol and Amikacin (96%), Genatmycin (90%), Amoxicillin (82%), Streptomycin (80%), Tetracycline (76%), Nalidixic acid (68%), Ampicillin 158%) and Sulfonamide (10%). The resistance was higest for Sulfonamide (76%) followed by Ampicillin (32%), Nalidixic acid (30%) and 6-20% for Gentamycin, Amoxicillin and Tetracycline.

Mara and Horan (2010**)** stated that Dysentery is initially managed by oral rehydration therapy. This treatment cannot be adequately maintained, hospital admission may be required for venous fluid replacement. It can usually be treated with antibiotics, such as ampicillin, sulfamethoxazole (also known as Bactrim or Septra), nalidixic acid and theuoroquinolone, ciprofloxacin. Shigella infections are less common in communities with accesspotable water and good sanitation. Hand washing practices have proven to an effective control measure even in area with poor sanitation

Islam *et al.,* (2010**)** determined Bacteriological quality of treated water of different sources by presumptive coliform count. In source-wise distribution of samples, 50% of mineral water, 87.5% of filtered water and 100% of tap water samples were exceeded the drinking water guideline value of WHO. 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 bio indicator 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%).

Gordon and Lowy (2008**)** found that *Staphylococcus arouse*s a versatile pathogen capable of musing a wide range of human diseases. However, the role of different virulence factors in the development of staphylococcal infections remains incompletely understood. Some clonal types well equipped to cause disease across the globe, whereas others are facile at causing disease 3: En o n g community members. In this review, general aspects of *staphylococcal* pathogenesis are addressed, with emphasis on methicillin-resistant strains *Staphylococcus aureus*is the almostxiversal cause of furuncles, carbuncles, and skin abscesses andworldwide is the most commonly identified agent responsible for skin and soft tissue infections. *S. aurous* skin and soft tissue infections frequently begin as minor boils or abscesses and may progress to severe infections involving muscle or bone and may disseminate to the lungs or heart valves (i.e. Endocarditic). Treatment of early infections consists of incising and draining the lesion, often aixampaniedby (3-lactam antimicrobial drugs, which are also effective against 13- hemolyticstre*ptoco*).

CHAPTER 3

MATERIALS AND METHODS

The research work was conducted during the period from August 2019 to November 2019 at the Microbiological laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The details outline of the materials and methods are given below:

3.1 Study area and Material

The water samples were collected from the different stored water in tanks at Hajji Mohamed Danish Science and technology university Male Halls in Danijpur and brought to the laboratory under aseptic condition for laboratory analysis. The research work was conducted during the period from July 2019to Nov 2019.

3.2 Collection of Sample from different Stored water in tanks at HSTU male halls

A total of 36 samples were collected from different stored water in tanks of human use, in HSTU male Halls in Dinajpur district. The samples were collected under aseptic condition with the help of pre sterilized test tube and immediately transferred into the microbiology laboratory, HSTU. From each sampling point 200 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.

3.3 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, electronic balance, syringe and needle, compound microscope, spirit lamps, match lighter, bacteriological loop, inoculums loop, autoclave machine, filter paper.

3.4 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-Vogesproskaur (MR-VP)

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

3.5 Bacteriological Media for culture

3.5.1 Liquid Media

Nutrient broth the medium was prepared by adding 13 g of nutrient broth powder to one litter 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/inch2) for 15 minutes. Peptone water This medium was prepared by dissolving 10 g of peptone water and 5g sodium chloride in 1littre of distilled water. The mixture was distributed in 5 ml volumes into clean bottles, and sterilized by autoclaving at 121°C (15lb/inch2) for 15 minutes.

3.5.2 Solid media

3.5.2.1 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 Petri dishes, these were incubated at 37°C for overnight to check their sterility and used for culture characterization (Carter, 1979).

3.5.2.2 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).

3.5.2.3 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 Petri dishes. 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 judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3.5.2.4 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).

3.5.2.5 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 45ºC and 50°C and distributed in Petri plates and allow the medium to solidify partially uncovered. (HIMEDIA and Leifson *et al.*, 1935).

3.5.2.6 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).

3.5.2.7 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 re suspended 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 4550°C. After solidification of the medium in the Petri dishes, these were incubated at 37°C for overnight to check their sterility and used for culture characterization (Carter, 1979).

3.5.2.8 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).

3.5.2.9 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).

3.5.2.10 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).

3.6 Reagents preparation

3.6.1 Methyl Red-VogesProskauer 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).

3.6.2 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.

3.6.3 Voges-Proskauer solution

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

3.6.4 Potassium hydroxide solution

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

3.6.5 Indole reagent

Kovac's reagent This solution was prepared by mixing 25 ml of concentrated Hydrochloric acid in 75 ml of a myl alcohol and to this mixture 5 grams of paradimethyl-aminohenzyldehidecrystals were added. This was then kept in a flask equipped with rubber cork for future use (Merchant and Packer, 1967).

3.6.6 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 (Na2HPO4, 12H2O), 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

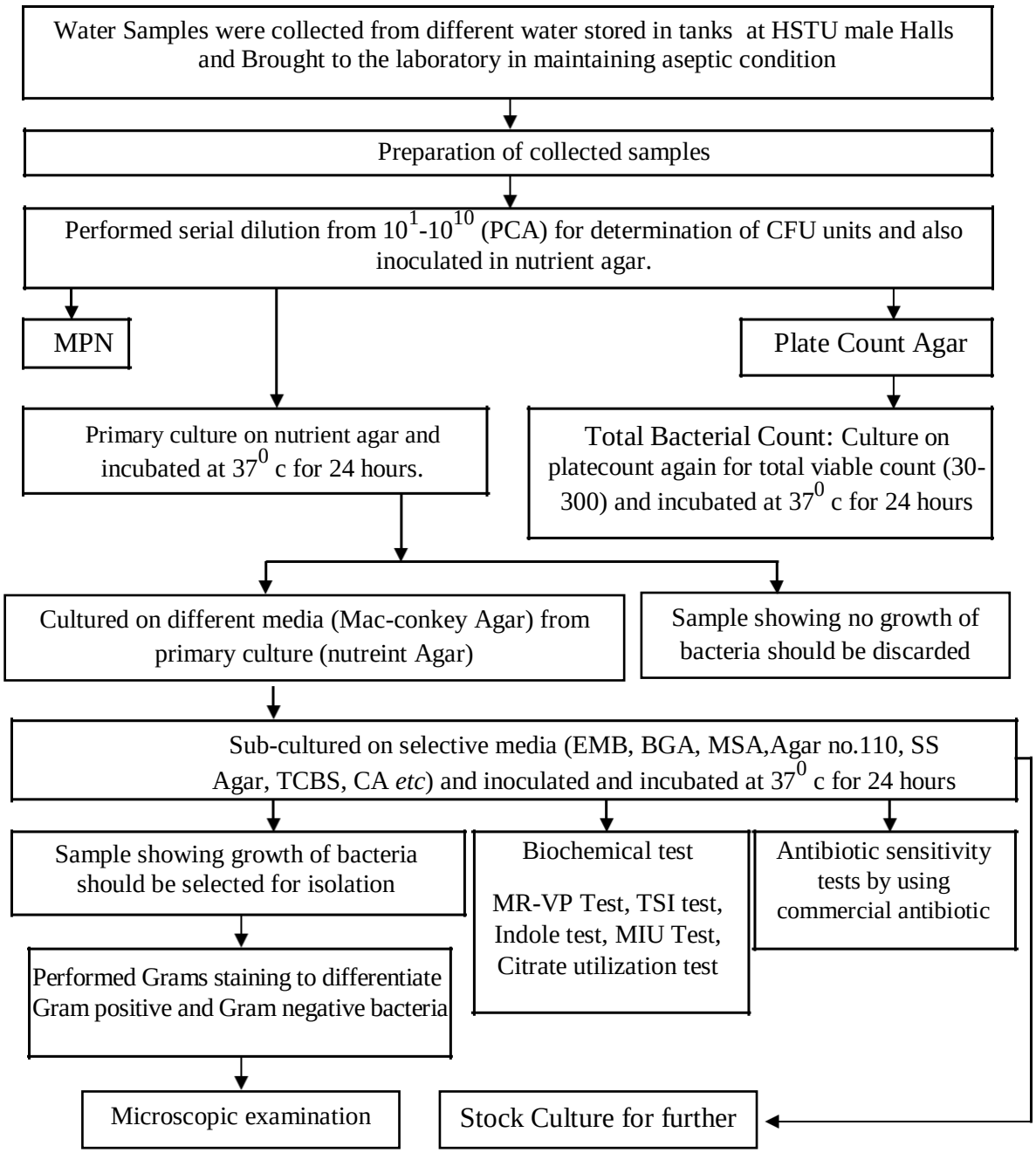
3.7 Laboratory preparation

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.

3.8 Experimental Layout

The present study was conducted into five phases; in first phase going to the selected area and collecting water, 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 use in future and Also PCR.

Experimental layout



3.9 Serial dilution of Sample

Each water tanks 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. 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.

3.10 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 37ºC 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:

$$\text{CFU} = \frac{\text{Colonies Counted} \times \text{Dilution}}{\text{Actual Volume of Sample plate}}$$

Calculation:

Colonies per plate=95

Dilution factor=106

Volume of dilution added to plate= 0.5 ml So, 1.9x108CFU/ml (Colony-forming units).

3.11 Total Coliform Count by MPN Method

Most probable number (MPN) test was used to detect the presence of coliforms in water sample. In presumptive MPN procedure, 15 lactose broth tubes were inoculated with the water samples. Five tubes received 10 ml of water, 5 tubes received 1 ml of water and 5 tubes received 0.1 ml of water. The number of tubes showing gas production and color change was compared to a standard table developed by American Public Health Association. The number of coliform was the MPN of coliforms per 100 ml of the water sample.

3.12 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 5 of 1.0 ml 5 of 10 ml 5 of 0.1 ml repeatedly sub-cultured by streak plate method (Cheesbrough, 1984) until the pure culture with homogenous colonies were obtained.

3.13 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: Inoculums was picked up with a sterile loop and spread on an area of the medium in the Petridis.

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.

3.14 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:

1. A loopful sterile distilled water was placed in the center of a clean sterile slide.
2. A small colony was picked up with a bacteriological loop and was mixed with distilled water of a slide
3. The colony was made to thin smear on a slide.
4. The smears were fixed by air driving.
5. 0.5% of crystal violet solution was then applied on the for two minutes
6. Then washout with clean water
7. Grams iodine was then added to act as mordant for one minute
8. Then washed out with clean water
9. Acetone alcohol was then added to decolorize for 1-2 seconds.
10. washed out with clean water
11. Safranin was as counter a stain and allowed for one minute.
12. Washed out with water.
13. 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.

3.15 Culture into differential media

3.15.1 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-fermented bacteria (pale color colony) were selected.

3.15.2 Culture on selective media

3.15.2.1 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.

3.15.2.2 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.

3.15.2.3 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.

3.15.2.4 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.

3.15.2.5 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).

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

Samples were inoculated int TCBS agar plates after incubation (370c for overnight) the yellow pigmented colonies indicated positive test for Vibrio species.

3.16 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.

3.16.1 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.

3.16.2 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.

3.16.2.1 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.

3.16.2.2 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.

3.16.2.3 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 37°C for 48 or 72 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.

3.16.2.4 VogesProskauer (VP) test

The test organisms were cultured in glucose phosphate peptone water for 72 hours at 37° C. 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 48 or 72 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.

3.16.2.5 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 of 28nitrogen).

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 37° C for 24 hours for incubation.Examined the tubes for the result.

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

3.16.2.6 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 ℃ in aerobic atmosphere.

Motility was observed by growth extending from the line of inoculums or diffuse turbidity of the medium. Non motile 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)

3.16.2.7 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°c 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 bubblesand black precipitation	K/A, GH_2S	Glucose fermentation only; Gas produced H2SProduced
Red/Yellow with blackprecipitation	K/A,H_2 S	Glucose fermentation only; H_2S Produced
Yellow/Yellowwithblack precipitation	A/A,H_2 S	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.

3.16.2.8 Antibiotic sensitivity tests

Bacterial susceptibility to anti-microbial agent was determined in vitro by using the standardized agar disc-diffusion method known as the Kirby Bauer (19), Labeled the covers of each of the agar plates with name of the test organisms was inoculated.

- 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.
- Allowed all culture plates to dry for about 5 minutes.
- Distributed the individual antibiotic discs at equal distance with forceps dipped in alcohol and flamed.
- 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.
- The plates were then inverted and incubated at 37°c for 24 hours.
- After incubation, the plates were examined and the diameter of the zones of complete inhibition was measured in mm.
- 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	30
4	Amikacin	AK	24
5	Chloramphenicol	C	30
6	Ceftraxone	CTR	30

source: CLSI- 2013

3.17 Reading Plates and Interpreting Results

After 24 hours of inoculation, each plate was examined if plate was satisfactory streaked, and the inoculum was corrected, the resulting zone of 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 zone of complete inhibition were measured. Including the diameter of the disc. Zones were measured to the nearest whole millimeter, using calipers or a 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, 2013).

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** ≥
Amikacin (GEN) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Staphylococcus spp.*	10 µg/disc	12	13-14	15
Tetracycline (TE) *Entrobacteriaceae* *Staphylococcus spp.*	30 µg/disc	13	14-17	18
Streptomycin(S) Entrobacteriaceae Staphylococcus spp	30 µg/disc	13	14-20	21
Ampicillin(AMP) Entrobacteriaceae. Acinetobacter spp. Pseudomonas aeruginosa	25 µg/disc	28	-	30
Chloramphenicol(C) Entrobacteriaceae Staphylococcus spp Streptococcus spp.	30µg/disc	13	14-17	18
Metradinazole(MT) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Acinetobacter spp.* *Staphylococcus spp***.**	5µg/disc	15	16-20	21
Moxifloxacin(MO) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Staphylococcus spp*	30 µg/disc	12	12-18	23
Erythromycin(E) *Entrobacteriaceae* *Pseudomonas aeruginosa.* *Staphylococcus spp*	30 µg/disc	13	13-20	30

3.18 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 -48 . The isolated organisms were given code name for convenience.

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)

The (TVC) of different stored water in tanks at Hajee Mohamed Danish Science and Technology University, Dinajpur are presented in Table

Table 1: Total viable count (TVC)

Place of Vendor	Dilution	Number of colony	Total viable count TVC)
Zia Hall (sample No 9)	10^{-1}	64	6.4x105 CFU/g
	10^{-2}	55	5.5x105 CFU/g
	10^{-3}	49	4.9x105 CFU/g
	10^{-4}	41	4.1x106 CFU/g
	10^{-5}	33	3.3x107 CFU/g
Tajudin Hall (sample No 9)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	80	8.0x105 CFU/g
	10^{-4}	66	6.6x106 CFU/g
	10^{-5}	58	5.8x107 CFU/g
Extension Hall (sample no 9)	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	97	9.7x105 CFU/g
	10^{-4}	70	7.0x106 CFU/g
	10^{-5}	63	6.3x107 CFU/g
Sheikh mujiburRahman Hall (Sample No - 9)	10^{-1}	51	5.1x105 CFU/g
	10^{-2}	45	4.5x105 CFU/g
	10^{-3}	41	4.1x105 CFU/g
	10^{-4}	35	3.5x106 CFU/g
	10^{-5}	29	2.9x107 CFU/g

TVC: Total viable count

The bacterial load was the highest in Extension Hall and Tajudin Hall (6.7×10^{7} CFU/g) According by Zia Hall and Sheikh Mujabir Rahman Hall

Table .2: 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 insingle	Gram (-)
6.	***Vibrio* spp**	Rod in shape	Arranged insingle	Gram (-)
7.	***Staphylococcus* spp**	Cocci in shape	Arranged incluster	Gram (+)
8.	***Micrococcus***	Cocci in shape	Arranged insingle	Gram (+)

4.2 Results of cultural examinations

Cultural characteristics of each type of bacteria isolated from different water sample were studied for the isolation, 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 sample and selective solid media for study. The individual cultural characteristics of bacterial isolates are presented in table 4.. The cultural characteristics of *E. Coli, Klebsiella spp, Salmonella spp, Shigella spp, Pseudomonas spp, Vibrio spp, Staphylococcus spp and micrococcus* exhibited on the media are presented in following figure.

Table 3: 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
08	*Micrococcus*	MacConkey	yellow pigmented colonies

Legends:

AGAR NO. 110, EMB = Eosin Methylene Blue, SS=*Salmonella Shigella,* TCBS=Thiosulfate-Citrate-Bile salts-Sucrose, CET= Cetrimide

4.3 Results of biochemical tests

The isolated organisms were confirmed by different biochemical tests. Following **table 4.5** represent the results obtain from different biochemical test for different stored water in tanks.

Table 4 Biochemical test

Biochemical test	***E. coli***		***Klebsiella spp***		***Salmonellaspp.***		***Shigella spp.***		***Pseudomonas spp.***		***Vibrio spp.***		***Staphylococcus spp***		**micrococcus spp**	
	Change of the media	**Results**	**Change of the media**	**Results**	**Change of the media**	**Results**	**Change of the media**	**Results**	**Change of the media**	**Results**	**Change of the media**	**Results**	**Change of the media**	**Results**	**Change of the media**	**Results**
MR test	Red color	Positive	Red color	Positive	Red color	Positive	Red color	Red color	No color change	Negative	No color change	Negative	red color	Positive	Red color	Positive
VP test	No color change	Negative	No color change	Negative	No color change	Negative	No color change	Negative	No color change	Negative	No color change	Negative	Red color	Positive	No color change	Negative
Triple sugar iron (TSI) test	Yellow color with gas	S-A, B-A, gas (+), H_2S (-)	Yellow color with gas	S-A, B-A, gas (+), H_2S (-)	S-Red, B-yellow	S-Al, B-A, gas (+), H_2S (+)	S-Red, B-yellow	S-Al, B-A, gas (+), H_2S (+)	S-yellow, B-yellow	S-A, B-A, gas (+), H_2S (-)	S-yellow, B-yellow	S-A, B-A, gas (+), H_2S (-)	S-Red, B-yellow	S-Al, B-A, gas (-), H_2S (-)	Yellow color with gas	S-A, B-A, gas (+), H_2S (-)
MIU test	Turbidity and changing of purple color of media	Positive	Turbidity and changing of purple color of media	Positive	No turbidity and no changing of color of media	Negative	No turbidity and no changing of color of media	Negative	S-A, B-A, gas (+), H_2S (-)	Positive	Turbidity and changing of color of media	Positive	No turbidity and no changing of color of media	negative	Turbidity and changing of purple color of media	Positive
Indole test	Pink rose color ring at the top of the media	Positive	Pink rose color ring at the top of the media	Positive	No color change	Negative	No color change	Negative	No color change	Negative	No color change	Positive	No color change	negative	Pink rose color ring at the top of the media	Positive
Citrate utilization test	No color change, No gas	Negative	No color change, No gas	positive	No color change	Negative	No color change	Negative	Prussian blue color	Positive	Prussian blue color	Positive	No color change	Negative	No color change, No gas	positive
Catalase test	No gas bubble	Negative	No gas bubble	Negative	Gas production	Positive	Gas production	Positive	Bubble produced	Positive	No Bubble produced	Negative	Bubble produced	Positive	No gas bubble	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).

4.4 Frequency of Isolated Bacterial Organism

Thirty four (36) of water sample were collected from different water stored in Tanks ; 9 water from Zia hall Water Tanks 9 sample from Tajudin Hall Water Tanks and 9 sample from Extension hall Water Tanks 9 water sample from Sheikh Majubir Rahman Water Tanks. From thirty four (36) water sample *Escherichia coli* was isolated 11(30%) highly frequent and followed by *Salmonella spp* 8(22%), *Shigella spp* 5(13.9%), *Klebsiella spp* 2(5.5%), *Vibrio spp* 1(2.8%), *Pseudomonas spp* 2(5.5%), *Staphylococcus spp* 5(13.9%) and *Micrococcus* 2(5.5%).

Table 5: Frequency of Bacteria isolated from water samples

Bacterial species	**Number of isolate bacteria from water stored in Tanks**				**Total (%)**
	Human water (36)				
	Tajudin Hall water (9)	**Zia Hall water (9)**	**Extension Hall (9)**	**Sheikh Majubir Rahman water (9)**	
Escherichia coli	4	2	2	3	11(30%)
Klebsiella spp	0	1	1	0	2(5.5%)
Salmonella spp	2	2	2	2	8(22%)
Shigella spp	0	1	2	2	5(13.9%)
Staphylococcus spp	1	1	1	2	5(13.9%)
Pseudomonas spp	1	1	0	0	2(5.5%)
Vibrio spp	0	0	1	0	1(2.8%)
Micrococcus	1	1	0	0	2(5.5%)
Total	9	9	9	9	36(44.4%)

Table 6 : Antibiotic Resistance Profile

Antimicrobial agents	*E-coli*			*Salmonella spp*			*Shigella spp*			*Staphylococcus spp*			*Klebsiella spp*			Speudomonas			Vibrio			Micrococus		
Name of Antibiotic	ZOI	Interp		ZOI	Interp		ZOI	Interp		ZOI	Interp		ZOI	Interp		ZOI	Interp		ZOI	Interp		ZOI	Interp	
		R	S		R	S		R	S		R	S		R	S		R	S		R	S		R	S
Moxifloxacine	21			22		S	35		S				30		S				30		S	30		S
Amikacin	25		S	21		S	20		S	25		S	23		S	23		S	25		S	28		S
tetracycline	30	R		7	R					8		R	7		R	8		R	6	R		7	R	
Chloramphenicol	26		S	27		S	20		S	21		S	25		S	24		S	27		S	26		S
Ampicillin	0	R					7	R					5	R		0	R					6	R	
Metradinazole	0	R		0	R		0	R		0	R		0	R		0	R		0	R		0	R	
Erythromycin	4	R		0	R																			
Streptomycin				28		S	26		S	25	S		25		S	22		S				26		S

Antibiotic Resistance Profile

[Note: ZOI= Zone Of Inhibition, Interp = Interpretation, S= Sensitive, R= Resistant, I= Intermediate

Table 8: Antibacterial profile of against *E. coil*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
E. coil	Amikacin(Ak)	22	S
	tetracycline (TE)	7	R
	streptomycin (S)	21	S
	Metradinazole (MET)	5	R
	Chloramphenicol(C)	18	S
	Ampicillin(AMP)	6	R

Note: TE tetracycline, C=Chloramphenicol, S= streptomycin, AK=Amikacin, AMP=Ampicillin, MET Metradinazole

I=Intermediate, S=Susceptible and R=Resistance

Table 9: Antibacterial profile of against *K. spp*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
K.spp	Metradinazole (MET)	5	R
	Amikacin(AK)	23	S
	Streptomycin (S)	20	S
	Chloramphenicol (C)	25	S
	tetracycline (TE)	8	R
	Moxifloxacine(MO)	25	S

Note: S =Streptomycin, C=Chloramphenicol, TE= Tetracycline, AK=Amikacin, AMP=, MO= Moxifloxacine MET= Metradinazole

I=Intermediate, S=Susceptible and R=Resistance

Table 10: Antibacterial profile of against *Staphylococcus spp,*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Staphylococcus spp,	Metradinazole (MET)	8	R
	Amikacin(AK)	23	S
	streptomycin (S)	30	S
	Chloramphenicol (C)	25	S
	Tetracycline TE	7	R

Note: MET = Metradinazole, C=Chloramphenicol, S =Streptomycin, AK=Amikacin, TE =Tetracycline,

I=Intermediate, S=Susceptible and R=Resistance

Table 11: Antibacterial profile of against *Pseudomonas spp,*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Pseudomonas spp	Ampcillin (AMP)	8	R
	Amikacin(AK)	23	S
	Metradinazole (MET)	5	R
	Chloramphenicol (C)	25	S
	tetracycline (TE)	5	R
	streptomycin (S)	28	S

Note: TE = tetracycline, C=Chloramphenicol, MET= Metradinazole, AK=Amikacin, AMP=Ampicillin, S= streptomycin (S)

I=Intermediate, S=Susceptible and R=Resistance

Table 12: Antibacterial profile of against *Vibrio sppspp,*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Vibrio spp	tetracycline (TE)	5	R
	Amikacin(AK)	23	S
	Metradinazole (MET)	6	R
	Chloramphenicol (C)	25	S
	Moxifloxacine(MO)	27	S

Note: MET = Metradinazole, C=Chloramphenicol, MO=Moxifloxacine, Ak= Amikacin, TE= tetracycline,

I=Intermediate, S=Susceptible and R=Resistance

Table 13: Antibacterial profile of against *Salmonella spp*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Salmonella spp	tetracycline (TE)	6	R
	Amikacin(AK)	23	S
	streptomycin (S)	25	S
	Chloramphenicol (C)	30	S
	Metradinazole (MET)	5	R
	|Erythromycin	4	R

Note: E= Erythromycin, C=Chloramphenicol, MET= Metradinazole, Ak=Amikacin, S= streptomycin, TET= tetracycline, I=Intermediate, S=Susceptible and R=Resistance

Note: R=Resistant, S=Sensitive, I=Intermediate

Table 14: Antibacterial profile of against *shigella spp*

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
shigella spp	Streptomycin(S)	22	S
	Amikacin(AK)	28	S
	Moxifloxacine(MO)	30	S
	Chloramphenicol (C)	10	S

Note: S = Streptomycin, C=Chloramphenicol, MO= Moxifloxacine, Ak=Amikacin, I=Intermediate, S=Susceptible and R=Resistance

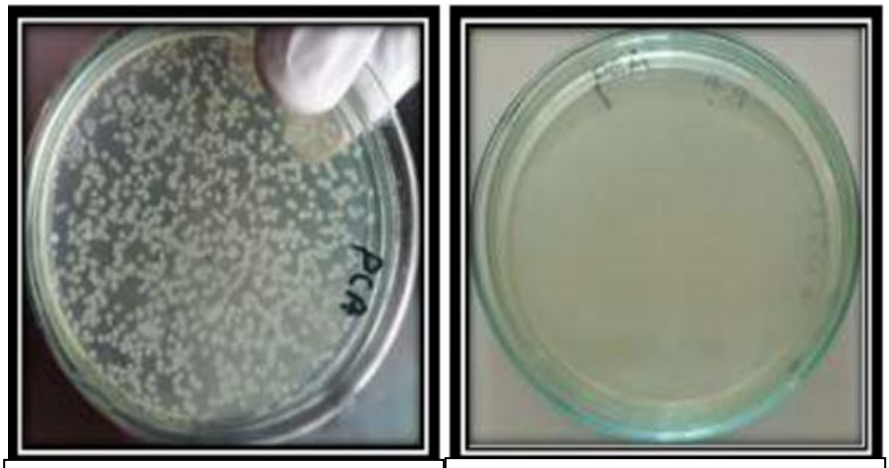
Note: R=Resistant, S=Sensitive, I=Intermediate

Table 15: Antibacterial profile of against micrococcus spp

Organism	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
micrococcus spp	Amikacin(Ak)	28	S
	Streptomycin(S)	21	S
	Metradinazole (MET)	6	R
	tetracycline (TE)	4	R
	Chloramphenicol(C)	28	S
	Ampicillin	5	R

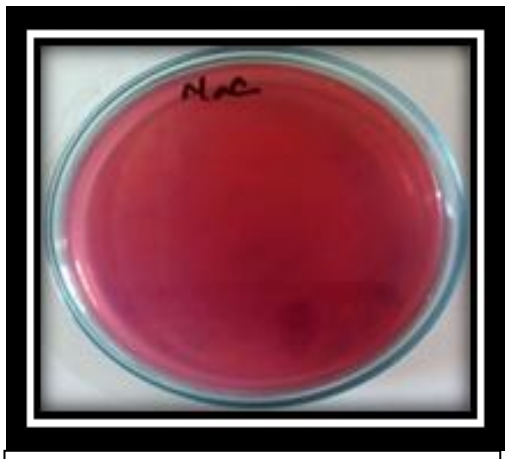
Note: TE = tetracycline, C=Chloramphenicol, S= Streptomycin, Ak= Amikacin, AMP=Ampicillin, MET= Metradinazole, I=Intermediate, S=Susceptible and R=Resistance

Note: R=Resistant, S=Sensitive, I=Intermediate



White Colonies Nutrient Agar | Nutrient Agar (control)

Fig. 4.1: Total viable count (TVC)



MacConkey control

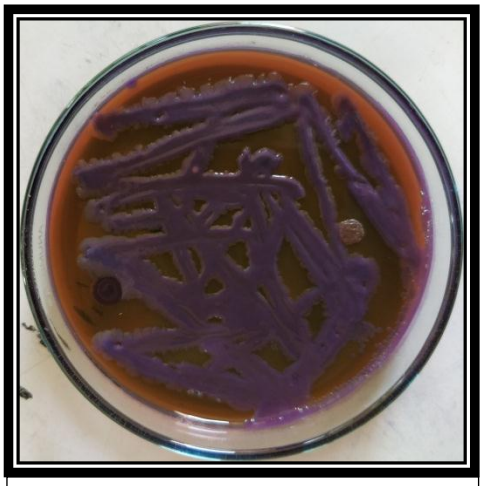
Pinkish colony of klebsiella Spp

Pink colony klebsiella

Control

Rose Pink golden colony of

Brilliant Green (Control)



Salmonella spp

SS Agar (Control)

Shigellaspp

Cetrimide Agar (Control)

Pseudomonas spp

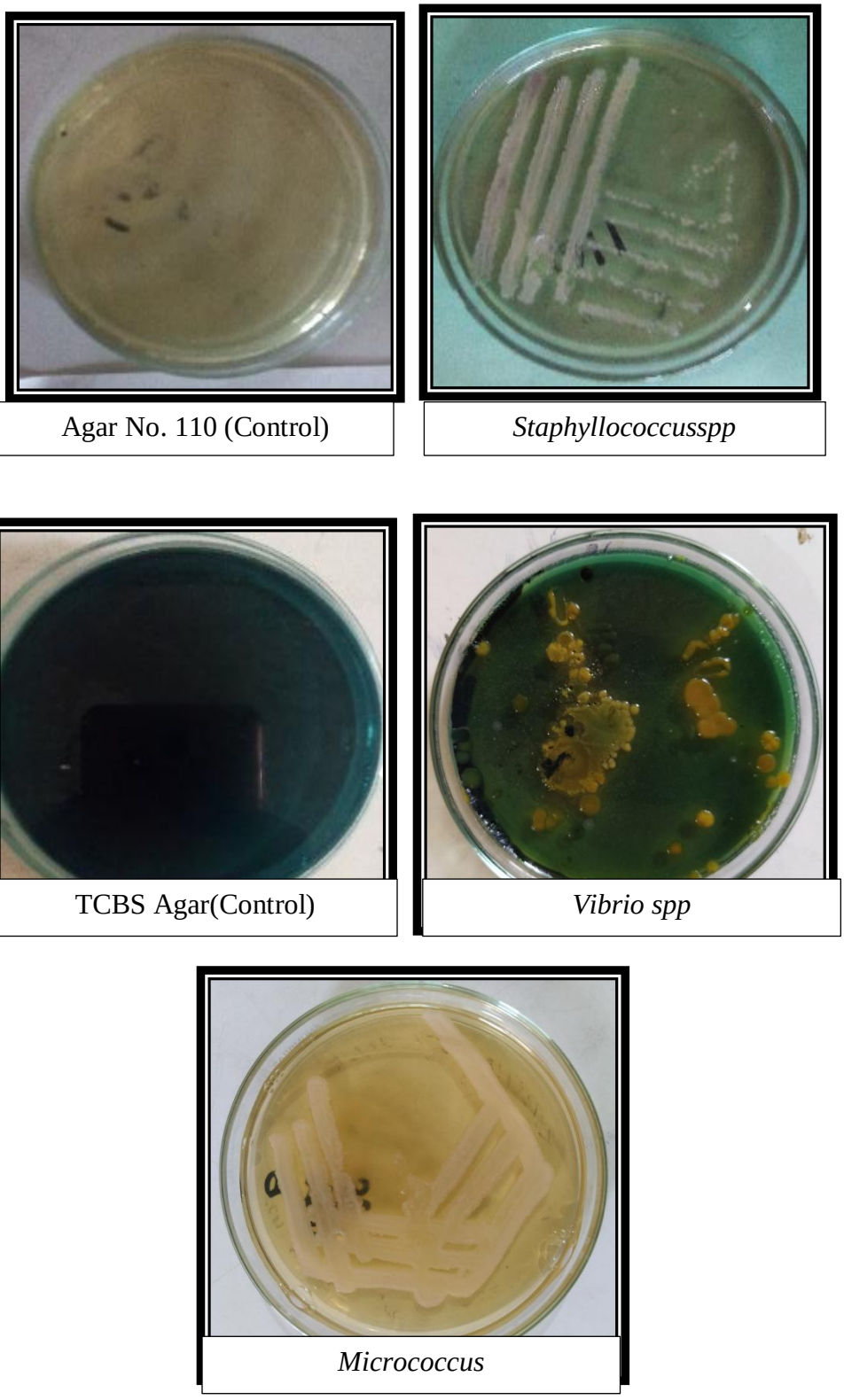


Fig. 4.2: Growth of Bacteria on Specific Culture Media

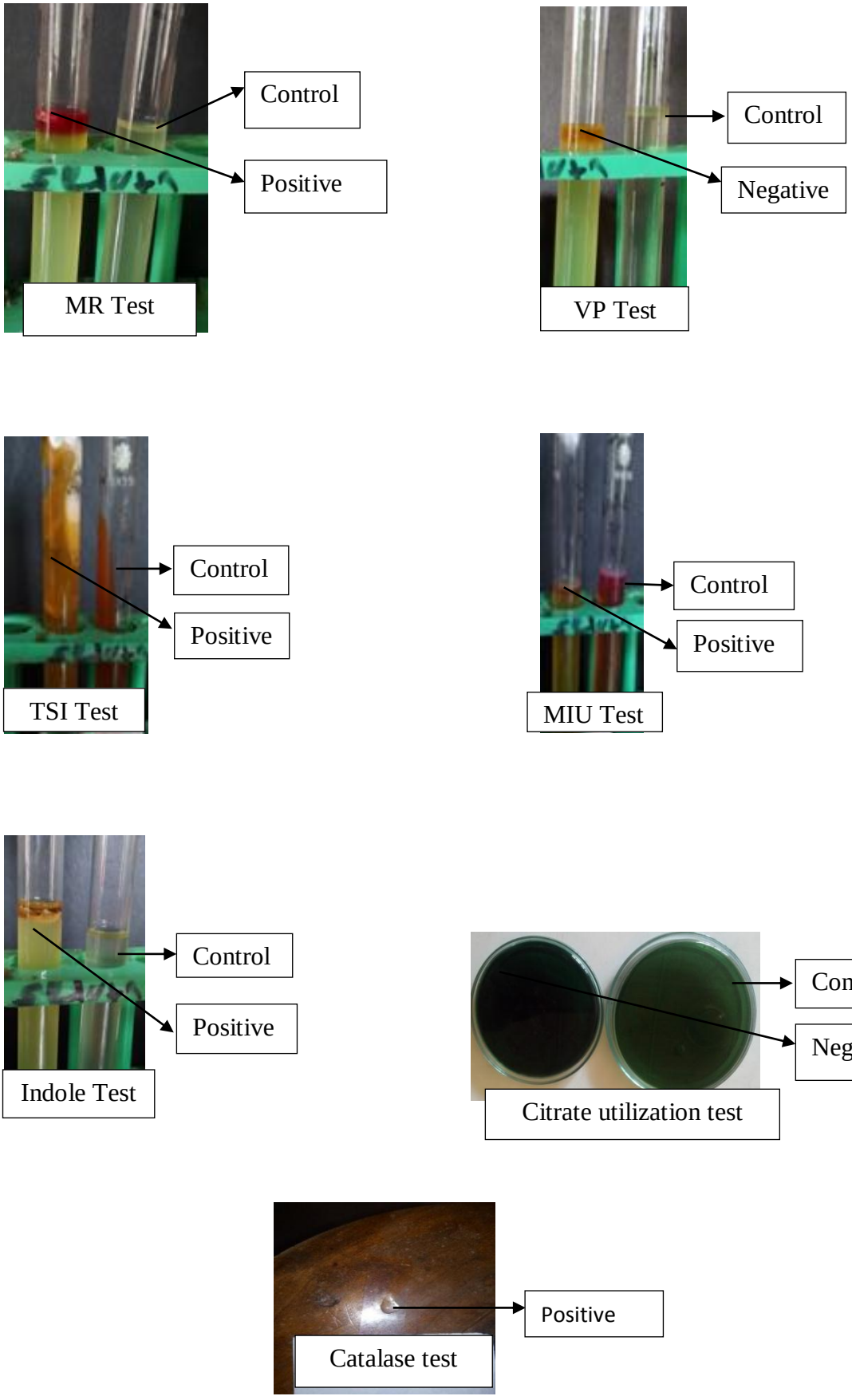


Fig. 4.3: Different biochemical test of *E. colispp.*

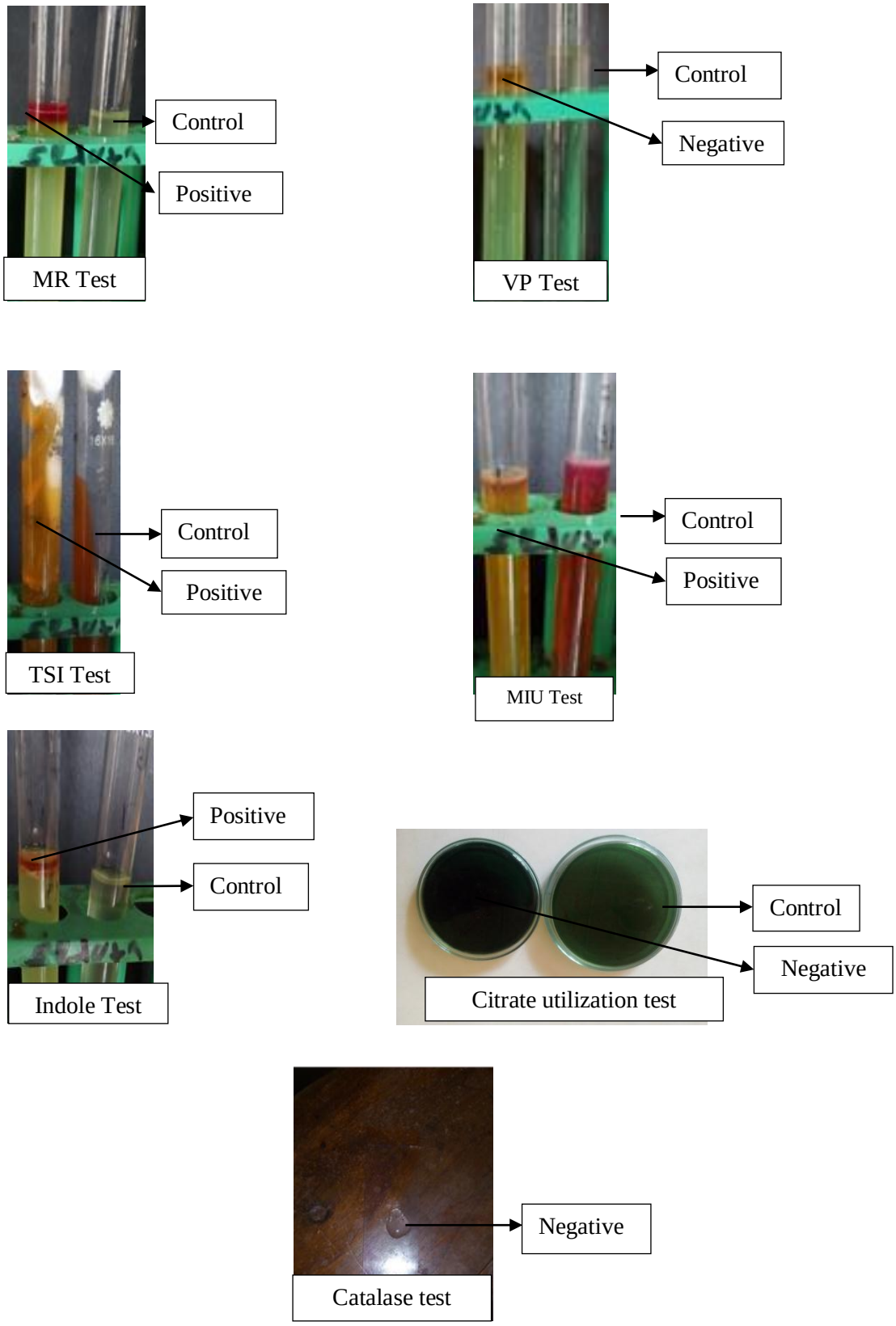


Fig. 4.4: Different biochemical test of *Klebsiella spp.*

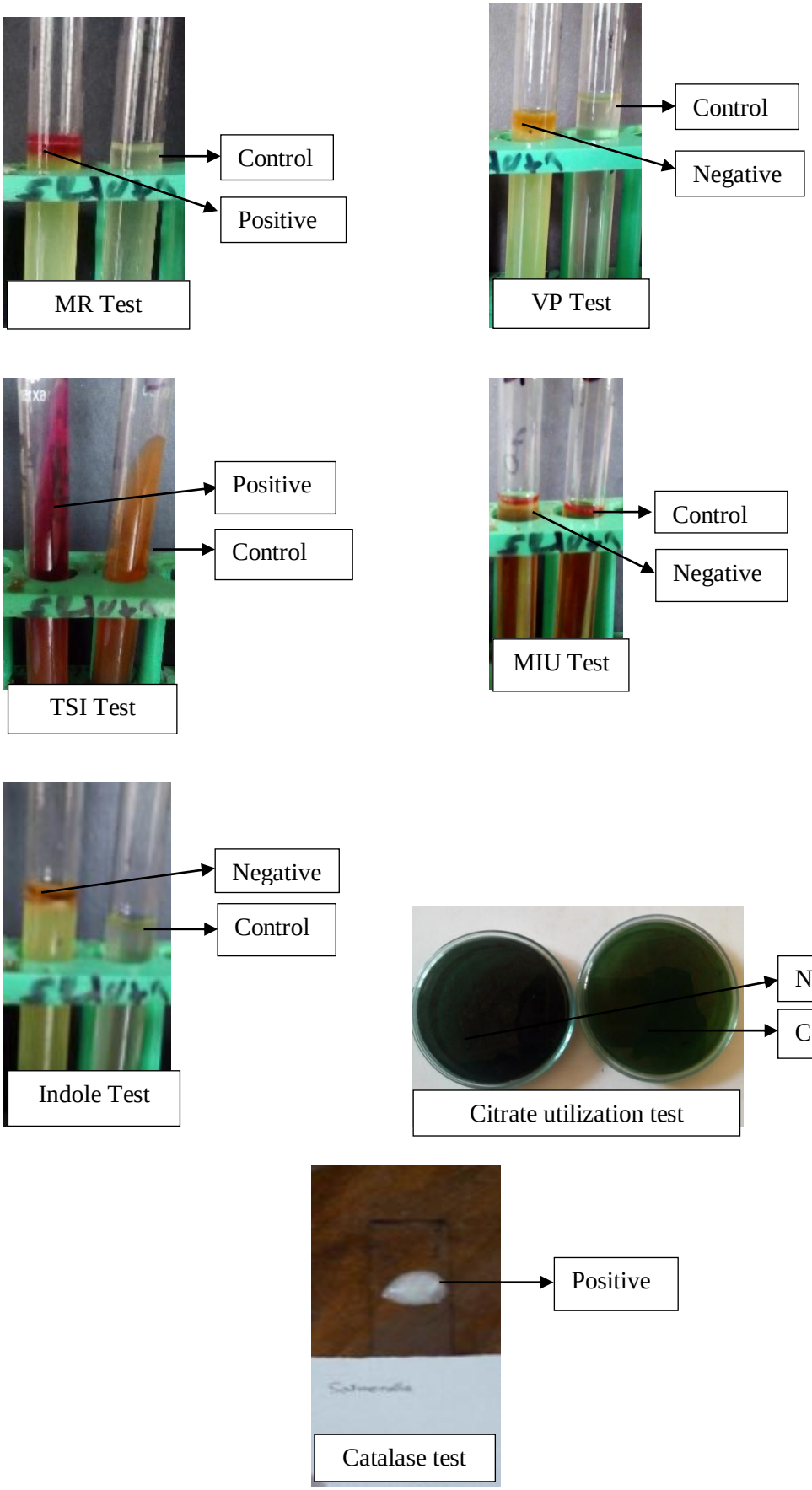


Fig. 4.5: Different biochemical test of *Salmonellaspp*.

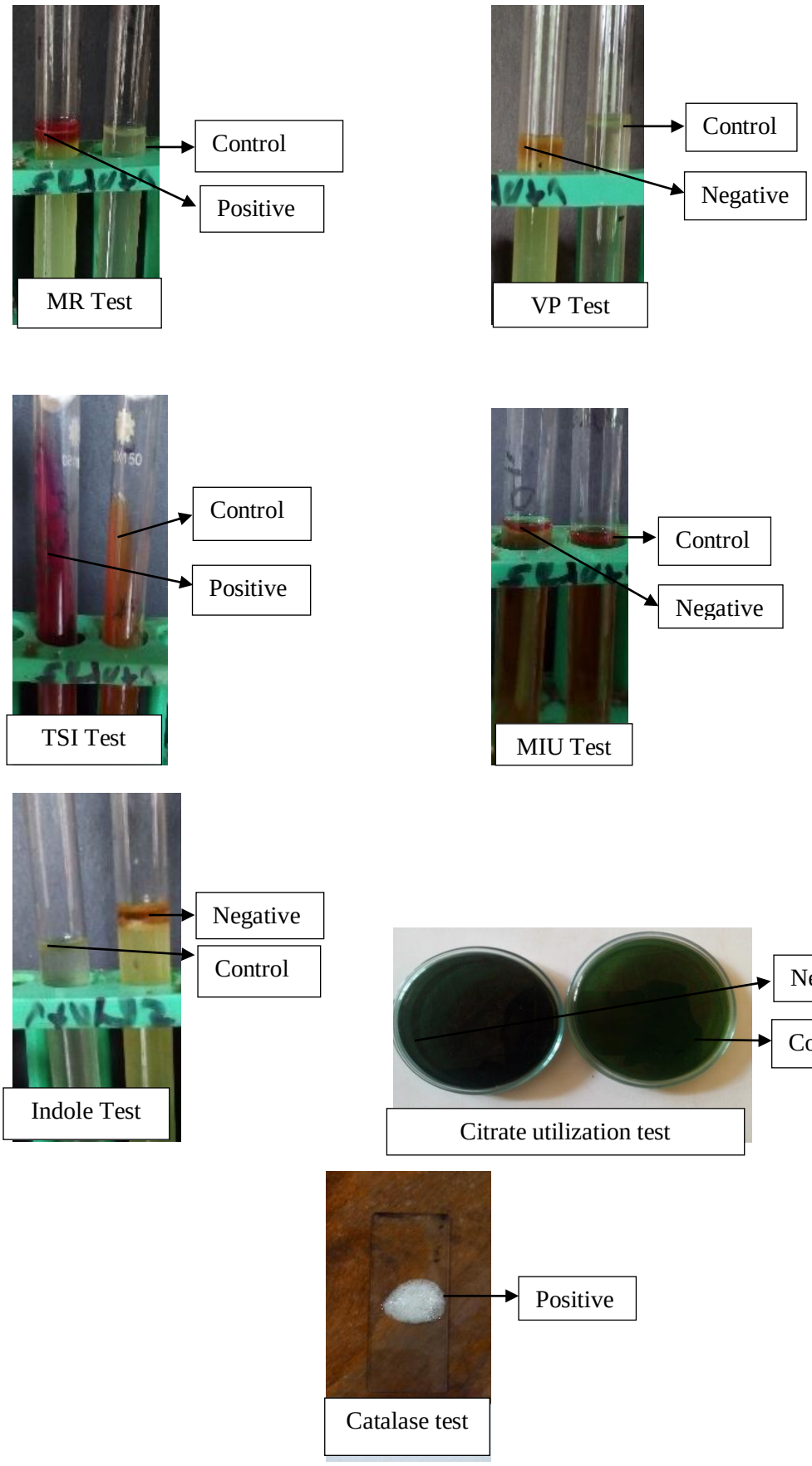


Fig. 4.6: Different biochemical test of *Shigella spp*.

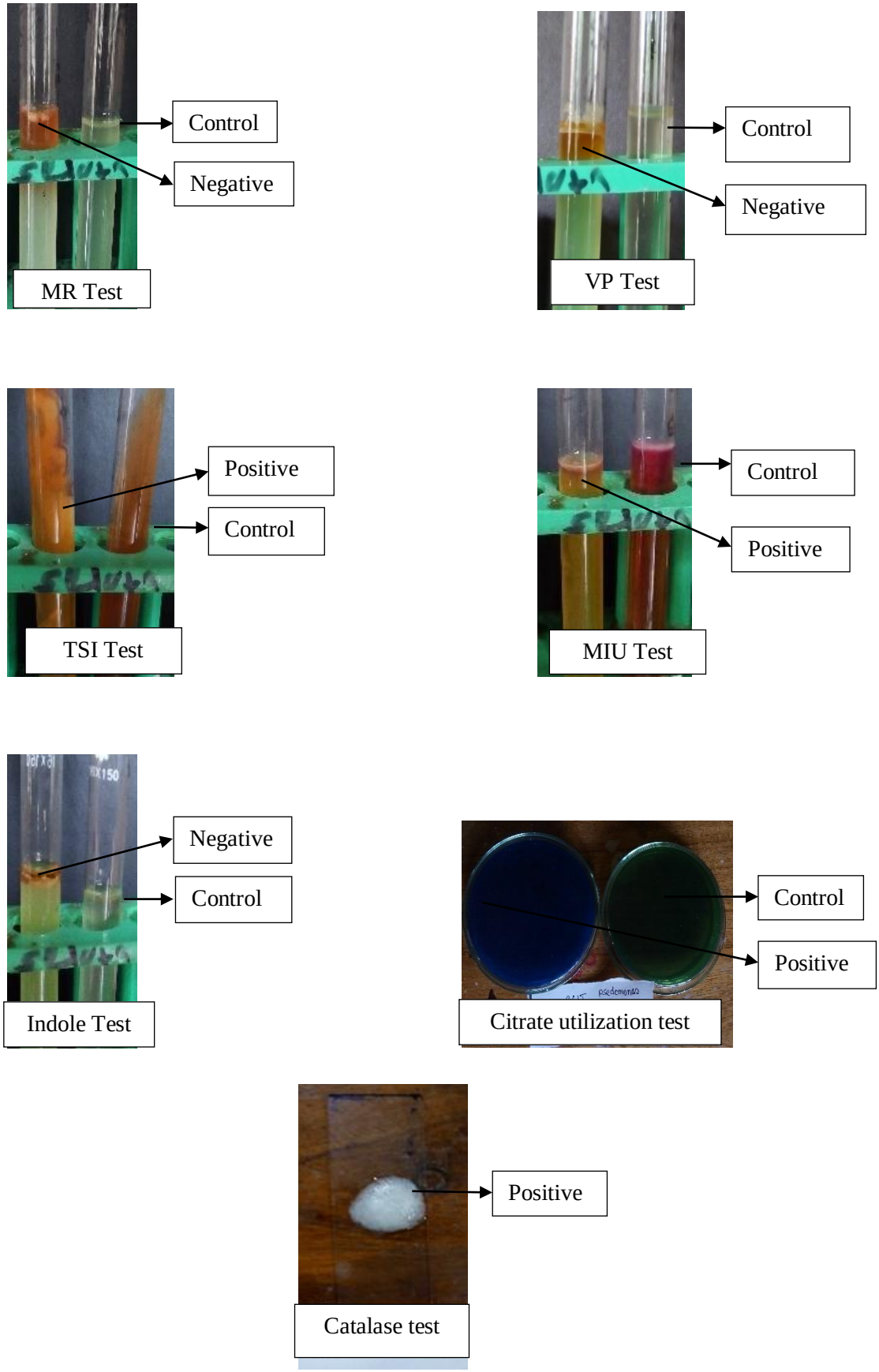


Fig. 4.7: Different biochemical test of *Pseudomonas spp*.

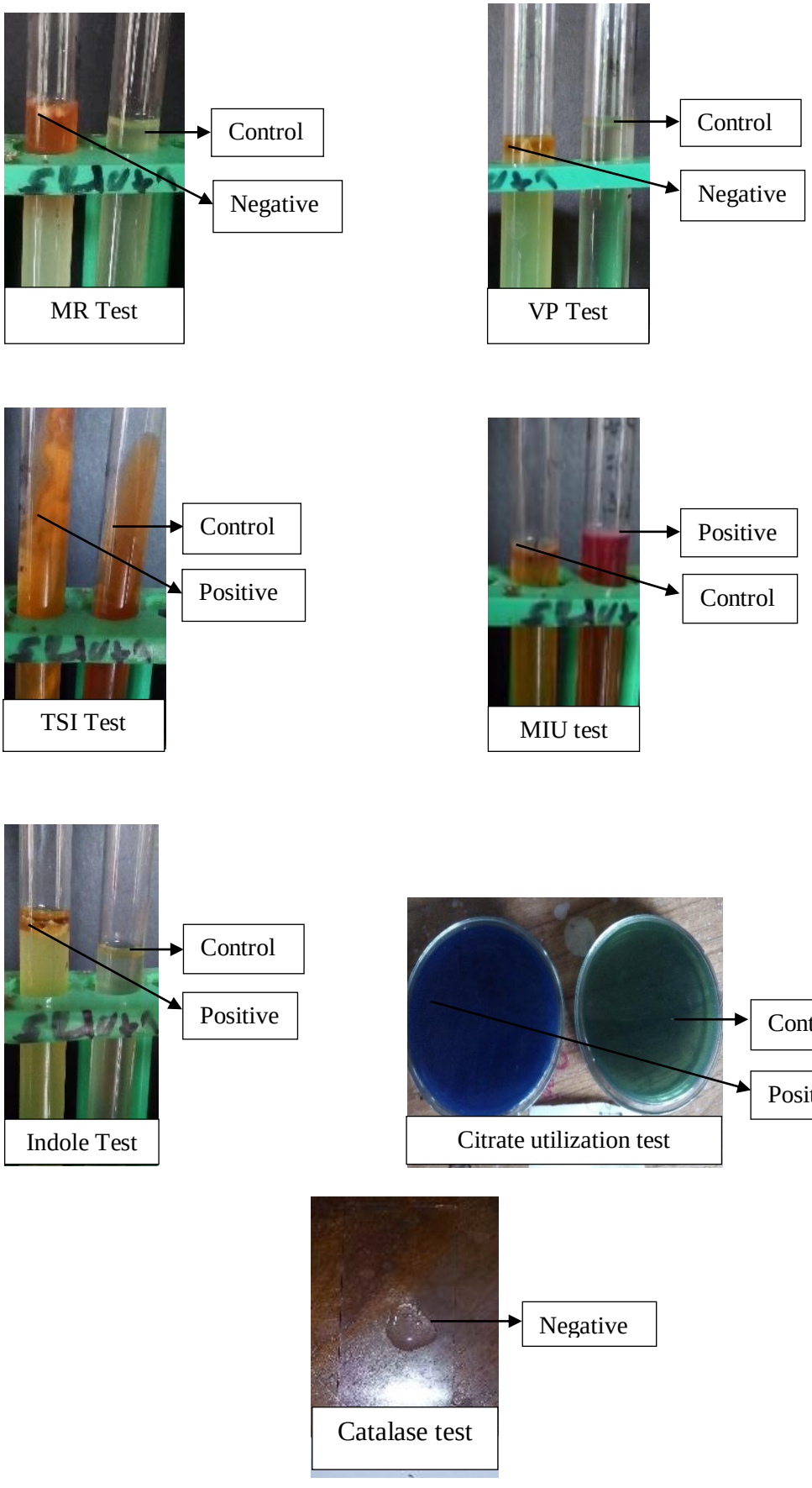


Fig. 4.8: Different biochemical test of *Vibrio spp*.

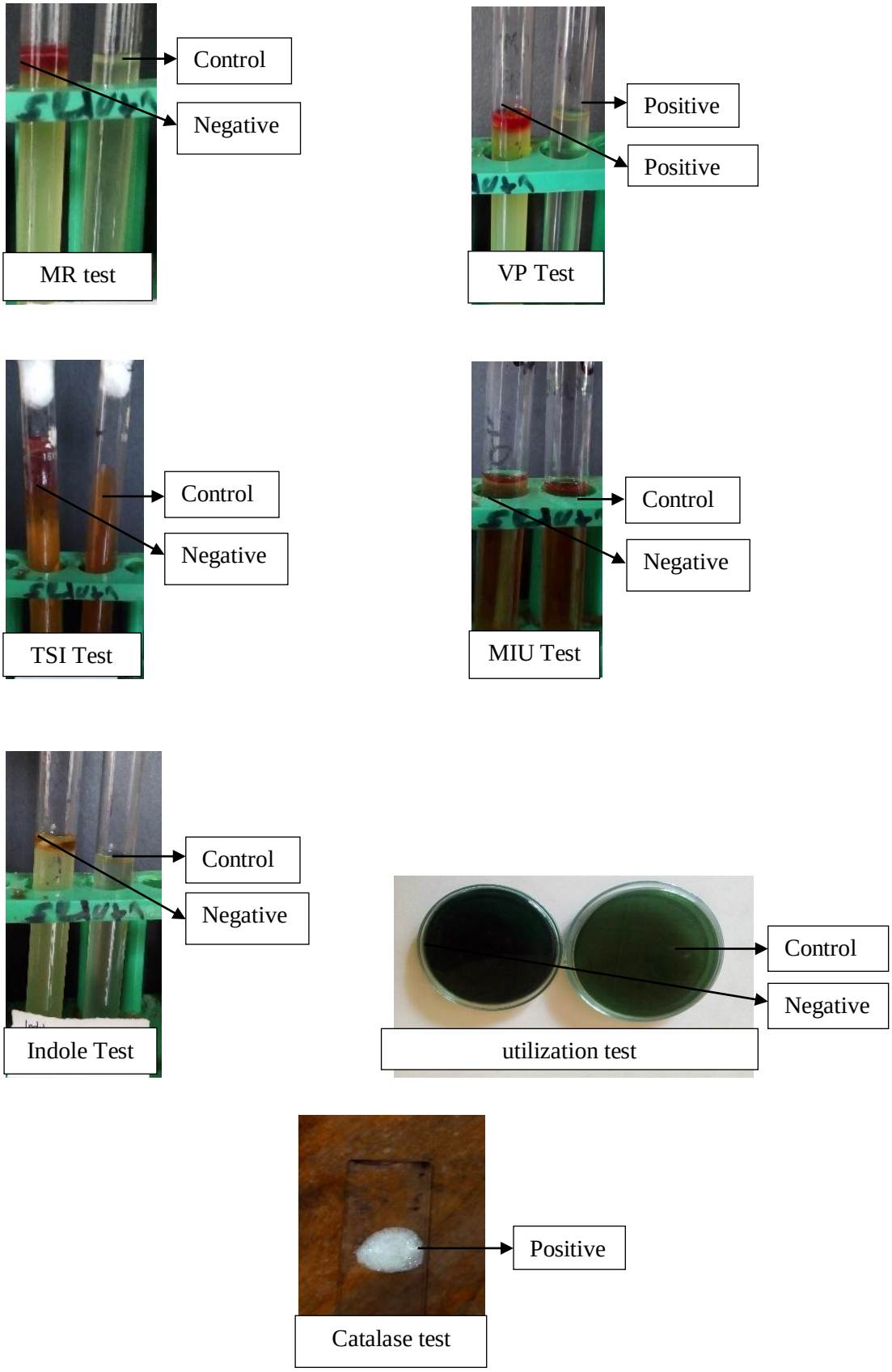


Fig. 4.9: Different biochemical test of *Staphylococcus spp.*

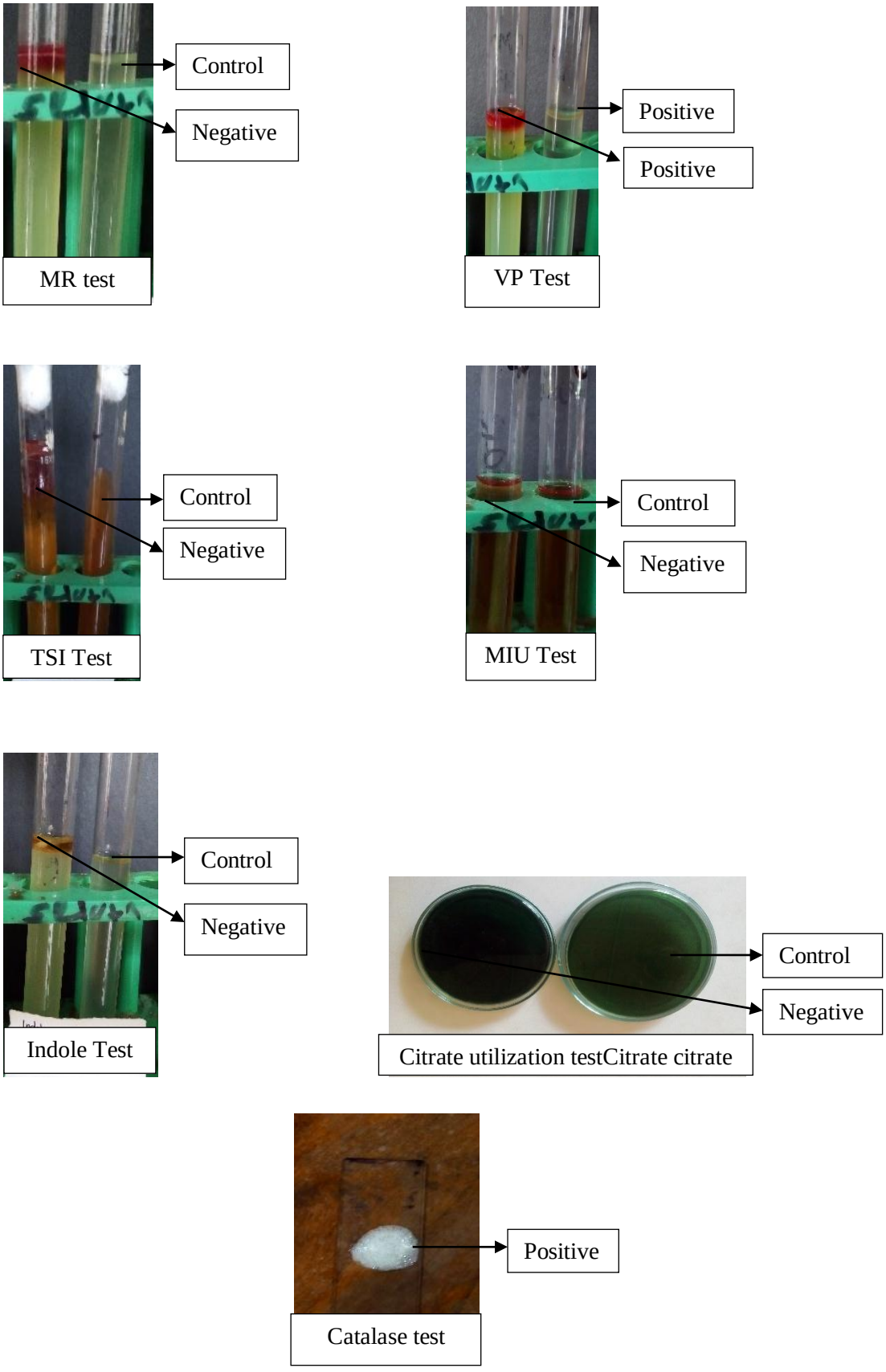


Fig. 4.10: Different biochemical test of micrococcus spp.

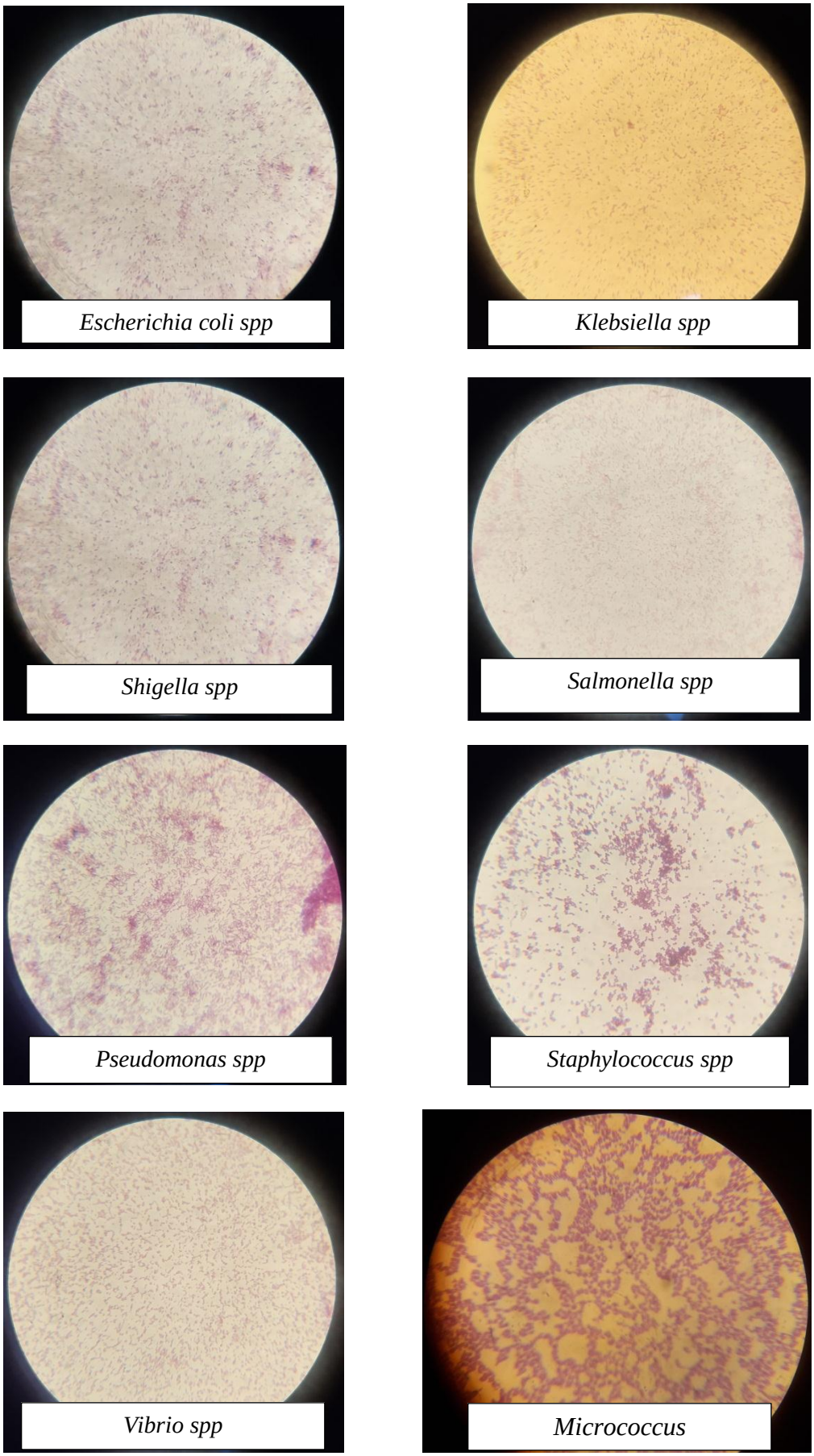


Fig. 4.11: Microscopic view of different bacterial species

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. Eight (8) isolates bacteria *E. coli, Klebsiella spp, Salmonella spp, Shigella spp, Staphylococcus spp, Pseudomonas spp., Vibrio* and *Micrococcus spp* were subjected to antibiotic sensitivity tests for water stored in tanks sample. The results of antibiotics sensitivity tests are presented by Tables

Fig. 4.12: Mueller Hinton Agar used in Antibiogram Study

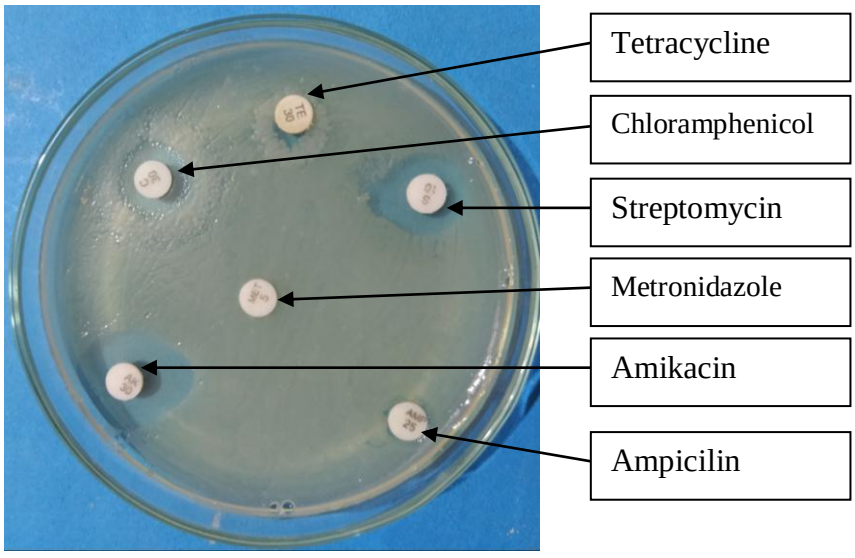


Fig. 4.13: Antimicrobial profile of against *E. coil*

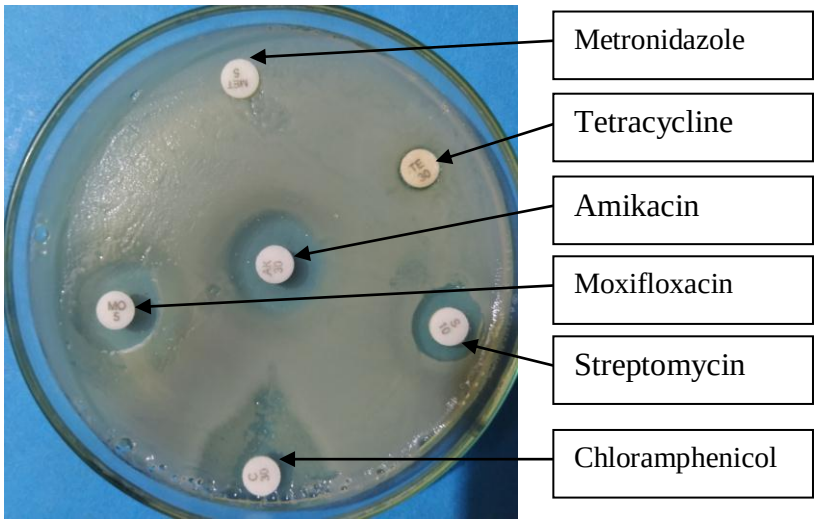


Fig. 4.14: Antimicrobial profile of against *K. spp*

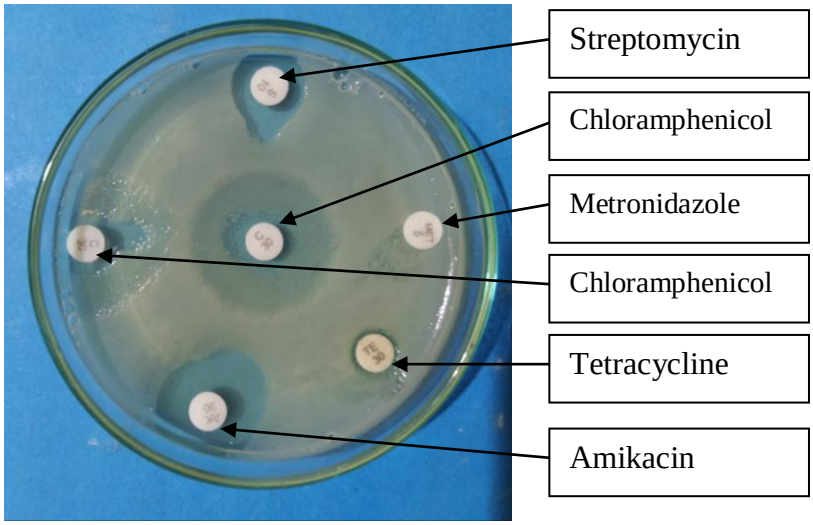


Fig. 4.15: Antimicrobial profile of against *Staphylococcus spp,*

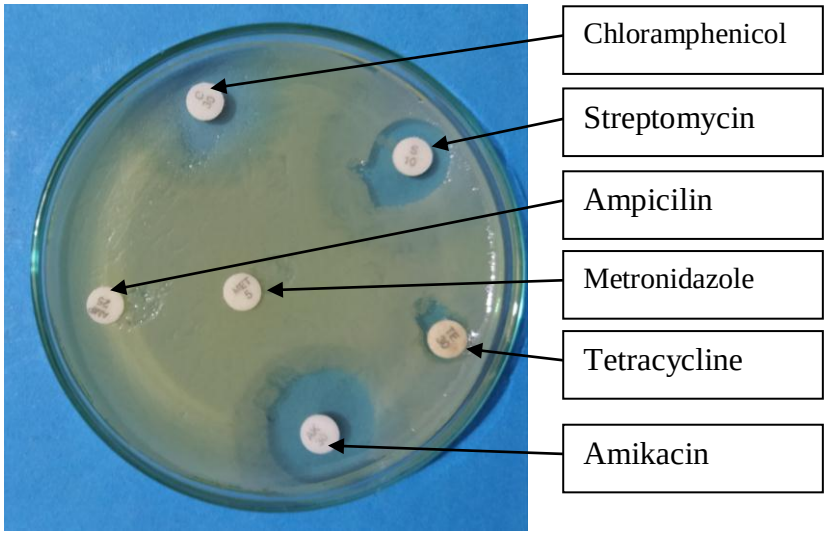


Fig. 4.16: Antimicrobial profile of against *Pseudomonas spp,*

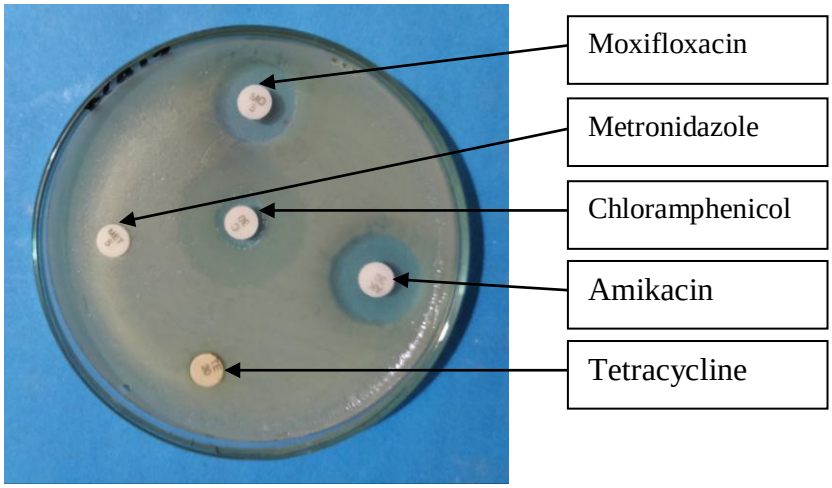


Fig. 4.17: Antimicrobial profile of against *Vibrio spp*

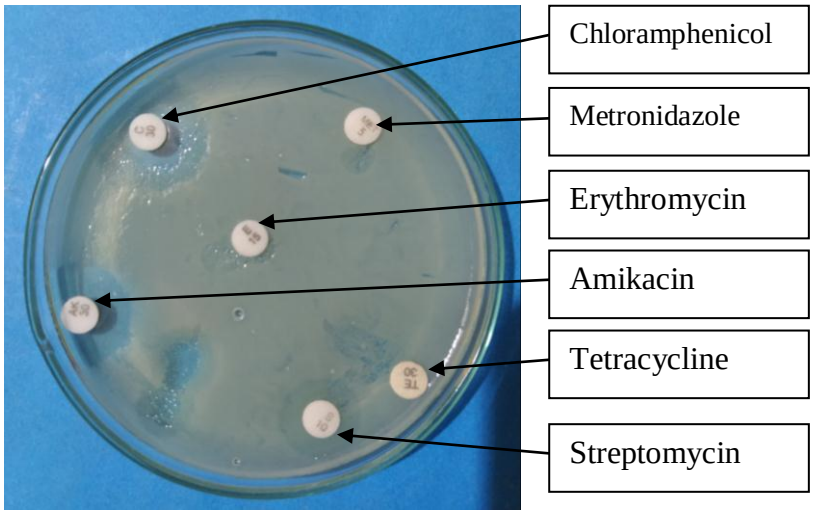


Fig. 4.18: Antimicrobial profile of against *Salmonella spp*

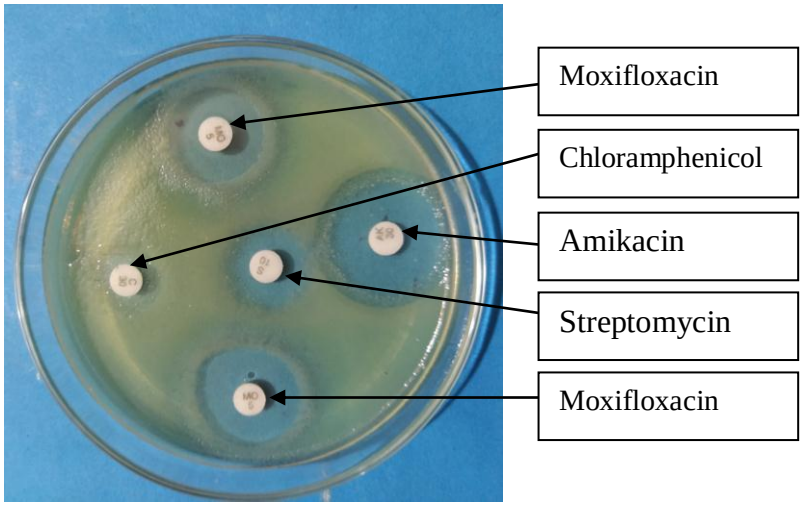


Fig. 4.19: Antimicrobial profile of against *shigella spp*

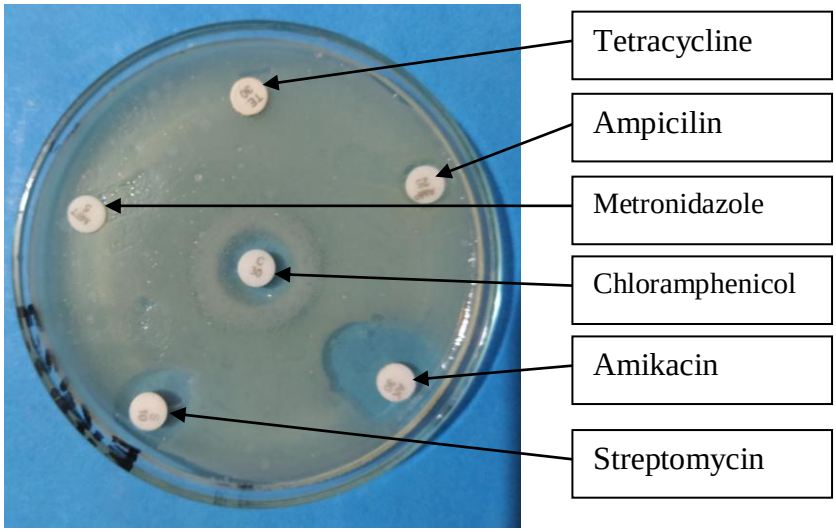


Fig. 4.20: Antimicrobial profile of against micrococcus spp

CHAPTER 5

DISCUSSION

The present study was conducted for bacteriological assessment of stored water in tanks at HSTU in some selected Tanks in Dinajpur district during the period from August to November 2019. In this study there were 36 samples and brought to the microbiology laboratory of the Department of microbiology, Hajee Mohammad Science and Technology University, Dinajpur for bacteriological examination, Isolation and identification of microorganisms were confirmed by their colony of different cultural media, staining characteristics and biochemical tests. The bacteriological media used in this study were selected according to **(**Buxton and Frazer, 1977).

the microbial load were significantly higher During first sample (before Dilution) the load of bacteria gradually Decreased when the Dilution is increase the first tube is high concentration of bacteria according the last tube in serial Dilution generally The bacterial load was the highest in Extension Hall and Tajudin Hall (6.7×107 CFU/g) According by Zia Hall and Sheikh Mujabir Rahman Hall, presented in table 1 this finding Nearbly Agrement in (Amaral *et al.*, 2004).

study isolation and Identification of bacteria In gram's staining, the morphology of isolated bacteria exhibited gram positive cocci in chains or in pairs and gram negative character was short rod arranged in single or paired, motile and non-motile 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 mostly found gram negative bacteria e.g. E. coli salmonella and gram positive bacteria e.g staphylococcus The positive bacteria gives purple color and Negative bacteria are gives in pink color present in table 2 this discussion is near by Figueras *et al.*, (2010)

Cultural characteristics of each type of bacteria isolated from different water sample were studied for the isolation, identification of various bacteriological media. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different sample and selective solid media for study. The individual cultural characteristics of bacterial isolates are presented in table 3 The cultural characteristics of *E. Coli, Klebsiella spp, Salmonella spp, Shigella spp, Pseudomonas*

spp, Vibrio spp, Staphylococcus spp and micrococcus found table 3 exhibited on the media are presented this information is same by (Buxton and Frazer, 1977).

Generally biochemical test is used to identify bacteria so the E,coli is methyl red MR positive changes color red and Also Voges-Proskauer (VP) is negative no color change MIU test E.coli is positive gives Turbidity and changing of purple color of media Salmonella also is MR test is positive and changes color in red and VP is negative no color change catalase is positive gas production, Pseudomonas spp. is MR,VP and Indol is negative no color change and Citrate utilization test is positive Prussian blue color produced, Staphylococcus spp MR,VP is positive changes color Red Indole test is negative no color change presented table 4 satisfied the statement of OIE, 2000

The study revealed that the prevalence of bacterial pathogens isolated from stored water in tanks samples were *Escherichia coli* was isolated 11(30%) highly frequency and followed by *Salmonella spp* 8(22%), *Shigella spp* 5(13.9%), *Klebsiella spp* 2(5.5%), *Vibrio spp* 1(2.8%), *Pseudomonas spp* 2(5.5%) and *Staphylococcus spp* 5(13.9%).and Micrococcus 2(5.5%) in The bacterial load was the highest in Extension Hall and Tajudin Hall According by Zia Hall and Sheikh Mujabir Rahman Hall this study evaluated the most Common Bacteria in stored water is E.coli and the lowest bacteria is Vibrio Spp present table 5 this finding supported by (Toze *et al.*, 1999).

In this study the antibiotic sensitivity test was performed by disk diffusion method accordingthe procedure described by Bauer *et al.* (1966) and Clinical Laboratory Standards Institution (CLSI, 2013).

In our study, it was observed Salmonella spp, E. coli, staphylococcus klebsiella spp are sensitive to Streptomycin, Chloramphenicol, Moxifloxacine, Amikacin, it was observed that isolates was resistance to tetracycline and Metradinazole, In the present study Klebsiella spp. showed were sensitive to Chloramphenicol, Amikacin, and also observed that klebsiella was resistance to Ampicilin and Amoxycillin On the other hand E. coli was sensitive to Chloramphenicol, Amikacin, and observed that E.coli was resistance to Ampicilin and Amoxycillin, Staphylococcus spp. was also sensitive to Ciproflaxin, Chloramphenicol, Amikacin, and it was observed that staphylococcus was resistance to Ampicilin and Amoxycilin the present table 6 this finding supported by Romya *et al.* (2013).

CHAPTER 6

SUMMARY AND CONCLUSION

The present study was conducted in order to isolate, identify and characterize the bacteria from stored water in Different Tanks HSTU malls Halls

The study revealed that the prevalence of bacterial pathogens isolated and identified from stored water samples were *Escherichia coli* was isolated 11(30%) highly friquent and followed by *Salmonella spp* 8(22%), *Shigella spp* 5(13.9%), *Klebsiella spp* 2(5.5%), *Vibrio spp* 1(2.8%), *Pseudomonas spp* 2(5.5%), *Staphylococcus spp* 2(5.5%) and micrococcus 2(5.5%).

In our study, it was observed Salmonella spp, E. coli, staphylococcus klebsiella spp are sensitive to Streptomycin, Chloramphenicol, Moxifloxacine, Amikacin, it was observed that isolates was resistance to tetracycline and Metradinazole,, In the present study Klebsiella spp. showed were sensitive to Chloramphenicol, Amikacin, and also observed that klebsiella was resistance to Ampicilin and Amoxycillin On the other hand E. coli was sensitive to Chloramphenicol, Amikacin, and observed that E.coli was resistance to Ampicilin and Amoxycillin, Staphylococcus spp. was also sensitive to Ciproflaxin, Chloramphenicol, Amikacin, and it was observed that staphylococcus was resistance to Ampicilin and Amoxycilin the present table 6 this finding supported by Romya *et al.*, (2013), If any contaminated in water wells and water pipes than stored water Also become contaminated. The presence of E. coli, Salmonella spp. Klebsiella spp., Shigella spp., Staphylococcus and pseudomonas causes health hazards to both Students and Staffs such as typhoid, dysentery, diarrhea, cholera and gastroenteritis. The antibiotic resistance properties of isolated bacteria can cause serious health hazards because of ineffective treatment of the sufferer's by the commonly described antibiotics.

Recommendation

- stored tanks Should be cleaned every six Month or once a year
- Tanks Should be Poured into Chlorine
- The Pipes that supply water to the Tanks should be protected any linkage to Avoid any Contamination of water

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