Microbiological Assessment on Water Quality Consumed By Community In Jeli, Kelantan

MOHAMAD SYAHID FARHAN BIN MOHD ZAKI

F18A0062

A thesis submitted in fulfilment of the requirements for

The Degree of Bachelor of Applied Science (Agrotechnology)

With Honours

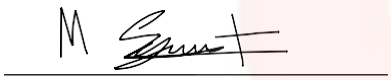
FACULTY OF AGRO-BASED INDUSTRY

UNIVERSITI MALAYSIA KELANTAN

2021

AUTHOR DECLARATION

I hereby declare that the work embodied in this report is the results of the original research and has not been submitted for a higher degree to any universities or institutions.

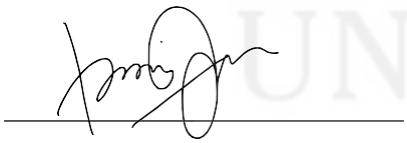


Name : MOHAMAD SYAHID FARHAN BIN MOHD ZAKI

Date : 2nd February 2022

I here certify that the thesis entitled "Microbiological Assessment on Water Quality Consumed by Community in Jeli, Kelantan" by Mohamad Syahid Farhan bin Mohd Zaki, matric number F18A0062 has been examined and all of the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:



Supervisor

Name : TS. DR. KHOMAIZON BINTI ABDUL KADIR PAHIRUL ZAMAN

Date : 3rd March 2022

Bacterial Assessment on Water Quality consumed by community in Jeli, Kelantan

ABSTRACT

The purpose of this study was to investigate bacterial assessment in the water consumed by community of Jeli, Kelantan. *E. coli, Salmonella spp.,* coliform was usually found in water which have been contaminated. Bacterial from ten different water source of two types of water sample of hill water and ground water were isolated and identified for possible bacteria.

The isolated bacteria from water sample were grown on selective agar of MacConkey agar, XLD agar and VRB agar were used to isolate *E. coli, Salmonella spp*. and coliform. The isolated colonies grown on the selective agar were identified using gram staining and biochemical test of catalase test and TSI test. Three water sample from ground water and three water sample form hill waters have been identified containing *E. coli. Salmonella* spp. were identified in three hill water sample and two round water sample. Three water sample from ground water and one from hill water sample were identified containing coliform in the water. One of ground water sample contain the highest total plate count with 2.44×10^4 cfu/mL. The presence of *E. coli, Salmonella spp*. and coliform in the water sample of hill water and ground water indicates the source or storage of the water has already been contaminated unknowingly and intentionally.

Keywords: Hill Water sample, Ground water sample, Contamination, faeces, Isolated bacterial, Total plate count

Penilaian Bakteria terhadap Kualiti Air yang digunakan oleh masyarakat di Jeli, Kelantan

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji penilaian bakteria dalam air yang digunakan oleh masyarakat Jeli, Kelantan. *E. coli*, Salmonella spp., Coliform biasanya ditemui dalam air yang telah tercemar. Bakteria daripada sepuluh sumber air berbeza daripada dua jenis punca air iaitu air bukit dan air bawah tanah telah diasingkan dan dikenal pasti untuk mendapatkan kemungkinan bakteria yang terdapat dalam sampel air tersebut. Bakteria terpencil daripada sampel air dikultur pada agar selektif iaitu agar MacConkey, agar XLD dan agar VRB digunakan untuk mengasingkan *E. coli*, Salmonella spp. dan koliform. Koloni terpencil yang tumbuh pada agar selektif dikenal pasti menggunakan pewarnaan gram dan ujian biokimia ujian katalase dan ujian TSI. Tiga sampel air daripada air bawah tanah dan tiga sampel air bukit telah dikenal pasti mengandungi *E. coli*. Salmonella spp. telah dikenalpasti dalam tiga sampel air bukit dan dua sampel air bulat. Tiga sampel air daripada air tanah dan satu daripada sampel air bukit dikenal pasti mengandungi koliform di dalam air tersebut. Satu sampel air tanah mengandungi jumlah kiraan plat tertinggi dengan 2.44 x 104 cfu/mL. Kehadiran *E. coli*, Salmonella spp. dan koliform dalam sampel air bukit dan air bawah tanah menunjukkan punca atau simpanan air tersebut telah pun tercemar secara tidak disedari dan disengajakan.

Kata kunci: Sampel Air Bukit, Sampel air tanah, Pencemaran, najis, bakteria terpencil, Jumlah kiraan plat

ACKNOWLEDGEMENT

BISMILLAHIRAHMANIRAHIM,

Firstly, I want to express my gratitude towards Almighty Allah for His blessing to give me a chance to complete this report with giving me a chance to learn and ability to complete the research. I am glad I am in a good condition all the way in completing this research.

Special thanks I dedicated to my supervisor for guiding me form the start. Without his untiring guidance, motivations, ideas, and constructive criticism, I would not be able to do complete my research. Thank you for the advice, knowledge, patience, and time that you spend to guide me to finish my research.

Next, I would like to thank all the person who involve directly or indirectly in my research. Such as people who help me in completing my lab test and made their valuable contributions to complete this dissertation. This dissertation is complete to meet the requirement for the award of The Degree of Bachelor of Applied Science (Agrotechnology) With Honours.

Finally, my endless gratitude to my beloved parents for always be there to support me through thick and thin with their endless love, care, courage and financial support. Biggest thanks to my friends and other people that contributed their ideas and motivation along doing this report. This dissertation might not be done without their help.

LIST OF SYMBOLS

L	Litre
mL	Millilitre
cm	Centimetre
℃	degree Celsius
cfu	Colony forming unit
H_2S	Hydrogen Sulfide
H_2O_2	Hydrogen Peroxide
g	Gram

TABLE OF CONTENTS

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Water quality differs from place to place and time to time. Most of the world's water is too salty for domestic uses and pollution from anthropogenic sources degraded the quality of freshwater, reducing its utility. While evaporation purifies water, the salts and contaminants left behind in the evaporated water contaminate the rainwater that returns. As the human population increases, the demand for goods and services that the population requires, or desires increase. As a result, there will be more water consumption and waste. Since the quantity of water cannot always be measured independently of its quality, water quality has become more important. Water quality is important for domestic, agricultural, and industrial water supplies, as well as fisheries and aquaculture production, aquatic recreation, and the health of ecosystems.

It is difficult to overlook the importance of trying to create a clean water source for humans around the world. Safe drinking water is a fundamental human right that is needed for good health and well-being. Historically, nearly all diseases such as cholera, measles, and typhoid fever were eradicated in societies where healthy and clean drinking water was made available in developing countries. Microbiological, chemical, radiological, and physical pollutants are the four types of contaminants that can be found in water. Bacteria, viruses, protozoa, and helminths are the four major groups of microbiological contaminants.

In countries like Malaysia, usually in the rural area such as in Jeli, Kelantan water quality had been an issue for so long. The community did not have a reliable water supply from the local government. Jeli district receives water supply from Pergau Reservoir Water Catchment from Air Kelantan SDN BHD, ground water and hill water from the hills nearby. According to the Malaysian Department of Environment, both the river and the lake have water quality indexes of 93.3 and 95.1, respectively, indicating that they are safe to drink, and the climate is healthy. The sampling of water for assessment was collected around Jeli area.

This study was carried out to investigate bacterial assessment on water quality consumed by community of Jeli, Kelantan. The results from this research provide information on the water quality whether it is contaminated with bacteria and safe to be consumed.

1.2 Problem Statement

The community in Jeli, Kelantan mostly were using groundwater and hill water for daily usage. It has become a concern as the source of water use were not treated and may contain harmful microorganism for human consumption. Waterborne diseases could be spread through untreated water consumption.

1.3 Hypothesis

H_o: The numbers of microbial count in water samples collected from Jeli, Kelantan community is at acceptable amount, $\leq 10^6$cfu/ml.

H_1: The numbers of microbial count in water samples collected from Jeli, Kelantan community is higher than acceptable amount, $> 10^6$cfu/ml.

1.4 Significance of Study

There are several significances in this study. This study will improve the community knowledge and concern on the water quality they consume using the microbiological count. Microbiological count can determine the water quality have the minimum limit microbial count and do not exceed the maximum limit.

In addition, the current water quality of Jeli community were consuming can be known thus could spread awareness to them about the importance of having a good water quality for daily usage and consumption.

1.5 Objective of Proposal

i. To determine the water quality, consume by community in Jeli, Kelantan based on microbial count.

ii. To identify types of microbes isolated from the collected water samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of Clean Water

The importance of water is crucial, but the idea of clean water is much more important. Per human on the planet is expected to need 20 to 50 litres of clean, healthy water every day (National Academy of Sciences,2016). This clean water can be used for bathing, cooking, and basic grooming and etc. There are a variety of harmful infectious agents that develop in contaminated/unsanitary water and can cause a variety of waterborne illnesses such as cholera, hepatitis, typhoid, and diarrhoea. Take, for example, diarrheal disorders caused by cholera, which is responsible for 1.8 million deaths worldwide (Khalifa & Bidaise, 2018).

A variety of factors apply to the availability of safe drinking water. Physical, demographic, geopolitical, and economic/socioeconomic status are among these considerations. The United Nations has designated clean water as a fundamental human right. In terms of sanitation, malnutrition, and education, the population suffers the most from a lack of access to safe water. The two key reasons holding communities apart from bright futures are diseases caused by drinking contaminated water and the time taken to fetch water due to access issues. As clean water becomes more available to populations, it is fair to believe that health will improve, resulting in less time spent due to illness. If there are fewer sick people, they are more likely to be at work, which will boost economic growth (The Water Project,2016).

Many of the harmful consequences of not getting access to clean water have already been addressed, but the list could go on and on. Take, for example, diarrhoea, which is regarded as the most serious public health issue associated with contaminated water. The figures are astounding: four billion cases of diarrhoea each year, 1.8 million deaths; nearly 90 percent of these deaths are said to be children under the age of five (Centre & Global Development, 2016).

Considering that water makes up most of the human body, it is easy to see why water is so important. Water is needed for basic human body functions such as sweating, digestion, brain activity, movement, filtration, and many others. A sufficient amount of water, for example, is needed for the body to clean itself by perspiration, defecation, and urination. Water deprivation impairs liver and kidney activity, and fluid excretion, such as urination, is impossible. In spite of defecation, the body hardens or softens stools based on the amount of water in them (Everyday Health, 2016).

2.2 Water Sources in Kelantan

As reported by the Economic Planning Unit in the 10th Malaysia Plan, Kelantan has only 59.5 % coverage of treated water in urban areas while 63.4 % in rural areas (Awang et al, 2020). Presently, there are a total of 24 well fields in Kelantan River Basin, 9 old and 15 new well fields which supply fresh groundwater obtained from a number of tube wells for their limited operation (Wan et al, 2014).

2.2.1 Water from Air Kelantan SDN BHD (AKSB)

Treated water supplied by Air Kelantan Sendirian Berhad was chosen by users as their main source for drinking (Awang et al, 2020). The total groundwater consumption constitutes about more than 45% (or 83 Mld) of the total water production in AKSB's water treatment plant and the demand of fresh groundwater for potable use will increase at a pace of 2.5% per year (NAHRIM, 2011). However not all of the homes get water supply from (AKSB).

2.2.2 Ground Water

In most areas of the world, the continuous growth of population density, urbanisation, irrigation, and manufacturing has increased pollution production, which leads to the degradation of surface water quality. This increases their reliance on groundwater supplies for their everyday needs, raising questions about their quality (Sefie et al, 2018). Since Jeli district is a remote district in Kelantan's interior, most residents in this area depend on groundwater supplies.

2.3 Contaminants in Water

The term "contaminant" in the Safe Drinking Water Act is described as any physical, chemical, biological, or radiological material or matter in water. As a result, the law describes "contaminant" narrowly as something other than water molecules. Any contaminants may be fairly expected to be present in drinking water in trace amounts. Any toxins in drinking water can be dangerous if consumed at certain amounts in drinking water, while others may be harmless. The existence of toxins does not always imply that the water is unsafe to drink. The Contaminant Candidate List contains only a subset of the universe of contaminants as described above (CCL). The CCL is the first standard of

assessment for untreated drinking water contaminants and could need further investigation of possible health impacts and amounts present in drinking water. Organisms in water are examples of biological contaminants. Microbes and microbiological contaminants are other terms for them. Bacteria, viruses, protozoans, and parasites are examples of biological or microbial contaminants.

2.3.1 Escherichia coli

Escherichia coli (abbreviated as *E. coli*) is gram -negative, rod-shaped bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhoea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (Division of Foodborne, Waterborne, and Environmental Diseases, 2014).

2.3.2 Salmonella spp

Salmonella spp. are a group of bacteria which reside in the intestinal tract of human beings and warm-blooded animals and are capable of causing disease. They are the second most common cause of bacterial foodborne illness in Ireland (*Campylobacter* spp. is the most frequent cause). They are facultative anaerobic Gram-negative rods. *Salmonella* spp. are members of the *Enterobacteriaceae* group (Food Safety Authority of Ireland, 2011).

2.3.3 Coliform

Coliform bacteria are organisms that are present in the environment and in the faeces of all warm-blooded animals and humans. Coliform bacteria will not likely cause illness. However, their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the faeces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for coliform bacteria. Most pathogens that can contaminate water supplies come from the faeces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for coliform bacteria. If coliform bacteria are found in a water sample, water system operators work to find the source of contamination and restore safe drinking water. There are three different groups of coliform bacteria; each has a different level of risk (Washington State Department of Health, n.d).

2.4 Waterborne Diseases

Currently, about 20% of the world's population lacks access to clean drinking water, and more than 5 million people die each year from illnesses caused by contaminated drinking water or insufficient sanitation. Per year, there will be 200 million fewer cases of diarrhoea and 2.1 million fewer deaths caused by diarrheal disease if we had clean drinking water and proper sanitation facilities (WHO 2001). A waterborne pathogen's target, like any other organism's, is to multiply and spread. The location of transmission and the method of distribution have significant consequences. Any bacteria spend the majority of their life in water and only come into contact with a host by chance. They are usually well suited to the low nitrogen concentrations as well as the physical, chemical, and biological conditions found in water (Ramírez-Castillo et al., 2015)

Water is their natural environment, and they can reproduce both in water and in the host. *Legionella spp*., *Pseudomonas aeruginosa*, several Mycobacteria species, and *N. fowleri* are examples of these so-called "environmental pathogens." These pathogens are distinguished by their ability to function in the absence of a host. Such waterborne pathogens (‘obligate pathogens') can only reproduce in an infected host. Replication usually takes place in the intestines of sick people (Nocker, Burr & Camper, 2014).

2.5 Water Treatment Process

Water treatment facilities are known to reduce the risk of parasites transmission in water According to Indah Water Konsortium (the country's main sewerage operator), the dominant wastewater treatment types are preliminary (removal of rags, rubbish, grit, oil, grease), primary (removal of settleable and floatable materials) and secondary treatment (biological treatment to remove organic and suspended solids) (Mat, Shaari & How, 2013).

Water treatment process generally may include are slightly different depending on location and the technology were used to undergo the treatment. However, the basic principle of the water treatment process is mostly the same. There are several processes which is coagulation or flocculation, sedimentation, filtration, disinfection, sludge drying, fluoridation and pH correction. The first process which is untreated water is treated with coagulation, liquid aluminium sulphate (alum), and/or silicone (raw water). As this is combined with water, the small bits of soil present bind together or coagulate. These dirt particle classes then combine to create bigger, harder particles known as flocs, which are easier to extract by settling or filtration (Hunter Water Corporation, 2011).

The second process, water and floc particles are moved into sedimentation tanks as they pass through the treatment process. The gradual movement of the water causes the heavy floc particles to fall to the ground. Sludge is the floc that gathers at the tank's bottom and is piped to dried lagoons.

The sedimentation stage is skipped in direct filtration, and the floc is eliminated solely by filtration. The third step, Water passes into a filter that is intended to separate contaminants from it. The filters are composed of layers of sand and gravel, as well as compressed anthracite in some situations. Filtration collects suspended impurities in liquids, improving disinfection efficiency. Backwashing is used to disinfect these filters

on a regular basis. The next step, water passes into a filter that is intended to separate contaminants from it. The filters are composed of layers of sand and gravel, as well as compressed anthracite in some situations. Filtration collects suspended impurities in liquids, improving disinfection efficiency. Backwashing is used to disinfect these filters on a regular basis. The next step is disinfection, water is disinfected until it reaches the delivery chain to eliminate disease-causing microorganisms (Hunter Water Corporation, 2011).

Chlorine is used as a disinfectant because it is extremely safe, and residual quantities should be retained to protect against any microbial pollution in the water treatment system. They will undergo sludge drying which is sedimentation and filtration absorb and settle solids in the water, which are then transferred to drying lagoons. After going sludge drying the water will undergo fluoridation where water fluoridation is the process of treating municipal water sources with a concentration of the free fluoride ion. This is set at an optimal degree in order to minimise dental decay (Hunter Water Corporation, 2011).

We are expected to fluoridate in compliance with the Fluoridation of Public Water Supplies Act 1957 of New South Wales. The final process is pH correction where aims to change the pH and stabilise the naturally soft water, lime is applied to purified water. This reduces rust in the delivery chain and in the plumbing of consumers (Hunter Water Corporation, 2011).

2.6 Water Analysis

Microbiological water analysis is a technique for estimating the amount of bacteria present in a sample of water and allowing for the recovery of microorganisms in order to classify them. The plate count is the form of inspection. The plate count method involves bacteria growing a colony on a nutrient medium until it becomes visible to the naked eye, allowing the number of colonies on a plate to be counted. The majority of laboratories use a system in which sample volumes of 100 mL or greater are vacuum filtered through specially designed membrane filters, which are then laid on nutrient medium inside sealed plates. To gain a complete count of the sample, a nonselective medium is used called a heterotrophic plate count. A selective medium may be used to extract a particular bacterial species (H. Kussmaul,2003).

In certain cases, research necessitates looking at predictor microorganisms. Indicator species are bacteria that are typically present in the human or animal gut, such as nonspecific coliforms, *E. coli*, and *P. aeruginosa*, and which, if observed, may indicate the presence of sewage. Specialty agars or research kits are used to identify certain cells.

2.7 Microbial Count

Total Plate Count (TPC) is a method of estimating the total number of microorganisms (mold, yeast, bacteria) in a material. The research begins with dilution phase of the sample until it reaches 10-5 dilution. Microbial total analysis was done by taking each 1 ml of dilution sample and inserted into 15-20 ml petri dish. The sample in the petri dish is lifted to freeze. The final stage is incubation by inserting a petri dish in an upside position into the incubator. Incubation is carried out at 36°C for 24-48 hours. Last done calculation and recording colony growth (Fahmi Arifan,2019).

2.8 Microbial Isolation

The pathogen bacteria colonies isolated from a water sample have grown on selective media. By observation done to colony morphology grown on selective media and compared with control plate, it is viable to identify which bacteria have been isolated from water sample collected. The selective media used in the experiment are XLD agar, MacConkey agar and VRB agar.

2.8.1 Xylose Lysine Deoxycholate (XLD) Agar

Xylose Lysine Deoxycholate (XLD) agar is a type selective growth medium use to grow from food and water for the isolation of *Salmonella* species. Taylor was the one who come about with the XLD agar to identify and isolate enteric pathogens and to assists the growth of enteric organisms. XLD Agar was designed specifically to oblige the growth and was demonstrate as a medium for the isolation of this *Salmonella* species. With a pH of approximately 7.4 with the indicator of phenol red, a bright pink or red appearance (Sagar Aryal,2022).

The fermentation of sugar lowers the pH and the phenol red turn to yellow. Salmonella is able to ferment the sugar xylose which producing acid while Shigella colonies impotent of doing so and therefore the colour remain red. Salmonella colonies will decarboxylate lysine, which would increase the pH once again to alkaline and mimicking the red Shigella colonies after using up the xylose supply. Salmonella produce H_2S by metabolizing thiosulfate that would leads to the formation of colonies with black centres and permits them to be distinguish from the similarly coloured of Shigella colonies (Sagar Aryal,2022).

2.8.2 MacConkey (MAC) Agar

MacConkey agar (MAC) is a bacterial culture medium named after bacteriologist Alfred T. MacConkey (1861-1931). MacConkey agar is a selective and differentiating agar that only grows gram-negative bacterial species; it can further differentiate the gram-negative organisms based on their lactose metabolism. The selective and differentiating properties of MacConkey agar enables utilization for both research and clinical applications. The fermentation of lactose produces organic acids, particularly lactic acid, which decreases the pH of the agar. MAC contains a pH indicator that turns pink under acidic conditions. Therefore, lactose-fermenting-gram-negatives (lactose-fermenters) will form pink colonies, while non-lactose fermenters will form off-white opaque colonies. Even within lactose-fermenters, species will show a varying rate of growth. The rate of growth is also a way to further differentiate organisms in the MAC medium. Lastly, some species that forms a capsule appear differently. Altogether, MacConkey agar only grows gram-negative bacteria, and those bacteria will appear differently based on their lactose fermenting ability as well as the rate of fermentation and the presence of a capsule or not (Benjamin Jung; Gilles J. Hoilat,2021). In this study Lac positive species was Escherichia coli.

2.8.3 Violet Red Bile (VRB) Agar

Violet Red Bile Agar is used for the enumeration of coliforms in food and dairy products in a laboratory setting. Violet Red Bile Agar is not intended for use in the diagnosis of disease or other conditions in humans and conforms to American Public Health Association (APHA). The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli. (Neogen ,n.d).

2.9 Microbial Identification

The colonies of bacteria grown on selective media were isolated from collected water sample was identified through gram staining and biochemical test include catalase test and TSI test.

2.9.1 Gram Staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram-positive and Gram-negative groups by colouring these cells red or violet. Gram-positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decolouring process. Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolorization, and counterstaining, usually with safranin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram-positive and Gram-negative bacteria, Gram-positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram-negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. (Monica Z. Brucker, n.d).

2.9.2 Biochemical Test

a. Catalase Test

An aerobic organism that detects the production of catalase enzyme in the organism can undergo a biochemical test named catalase test. A common enzyme which found in all living beings who could survive in oxygen and catalyses the decomposition of H_20_2 would release gas of H_2O and O_2 called catalase enzyme. In pathogenic organism catalase was essential as it could defend the organism from oxidative damages due to the reactive oxygen species. The bactericidal effects of hydrogen peroxide will be neutralized, and the bacteria concentration correlated with the pathogenicity of the organism. The catalase test has been extensively used over the years as it permits to distinguish catalase-positive organisms like staphylococci from catalase-negative species like streptococci. The presumptive characterization of most bacteria catalase test was very useful. Under the aerobic condition, 3% H_2O_2 is used, whereas 15% H_2O_2 is used under anaerobic conditions (Anupama Sapkota, 2020).

The catalase will accelerate the breakdown of hydrogen peroxide, H_2O_2 into water and oxygen (). Below was the chemical reaction breakdown of hydrogen peroxide:

$$2H_2O_2 + \text{Catalase} \rightarrow 2H_2O + O_2$$

The catalase test was done by collecting a small amount of organism from a well-isolated 18- to 24-hour colony with a sterile inoculating loop and placed onto the microscope slide. A drop of 3% H_2O_2 onto the organism on the microscope slide by using a dropper or Pasteur pipette. The formation of bubbles is observed against a dark background to enhance readability (Anupama Sapkota,2020).

b. TSI Test

The Triple Sugar Iron (TSI) test is a microbiological test named for its ability to test a microorganism's ability to ferment sugars and to produce hydrogen sulfide. An agar slant of a special medium with multiple sugars constituting a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate is used for carrying out the test.

All of these ingredients when mixed together and allowed solidification at an angle result in a agar test tube at a slanted angle. The slanted shape of this medium provides an array of surfaces that are either exposed to oxygen-containing air in varying degrees (an aerobic environment) or not exposed to air (an anaerobic environment) under which fermentation patterns of organisms are determined (Sagar Aryal, 2019).

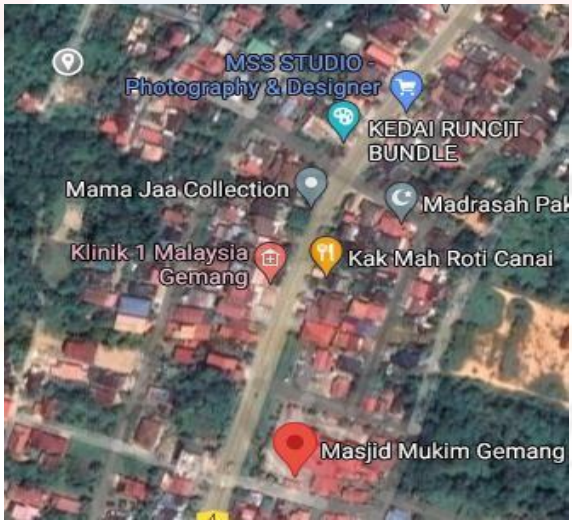
CHAPTER 3

MATERIALS AND METHODS

3.1 Material

The materials used were two types of collected water which is underground water (well water) and hill water (residential using hill water source). Each point of sample collection was recorded as well as its coordinate, time and date. The radius of location for collected water sample were shown in the satellite image below:

Figure 3.4.2 Shows the Location of Residential for Water Sampling in Gemang, Jeli.



The list of coordinates of two types of water sample were shown on the Table 3.1 below:

Table 3.1: List of coordinates collected water sample

Type of water source	Water sample	Coordinates
Ground water (Well Water)	I	5°45'43.9"N 101°51'57.1"E
	II	5°45'44.5"N 101°51'57.5"E
	III	5°45'49.9"N 101°52'00.0"E
	IV	5°45'29.5"N 101°51'54.7"E
	V	5°45'38.4"N 101°51'50.7"E
Hill water (residentials using hill water source)	I	5°45'39.3"N 101°51'55.9"E
	II	5°45'32.2"N 101°51'55.1"E
	III	5°45'05.7"N 101°51'50.6"E
	IV	5°45'31.6"N 101°51'47.0"E
	V	5°45'42.9"N 101°51'49.2"E

The list of chemicals used in this experiment was sodium hydroxide, Nutrient agar powder (HIMEDIA®), Xylose lysine deoxycholate (XLD) agar powder (Merck). MacConkey agar powder (HIMEDIA®) and Vile Red Bile (VRB) agar powder (Merck).

The list of materials was used in this experiment was 50 mL centrifuge tubes (Falcon™ & Biologix®), 15 mL centrifuge tubes (Falcon™ & Biologix®), petri dishes, paper towel roll, A4 paper, black colour manila card, aluminum foil and zip lock bag.

3.2 Equipment

The list of equipment was used in this experiment was laminar air flow cabinet (Azteclab), colony counter (Funke Gerber), 37 ℃ incubator (Lab Companion) and microscope (Leica) autoclave machine (Hirayama).

3.3 Methodology

3.3.1 Sample collection

Two type of water sample of high potential containing bacteria were collected from residential of Jeli, Kelantan which is underground water (well water) and hill water (residential using hill water source). Total number of ten water sample of five type each of mention above were collected.

3.3.2 Growth Media Preparation

a) Nutrient Agar

The agar mixture was prepared, 28 g of nutrient agar powder suspended into 800ml of distilled water then filled until 1L with distilled water in 1L media bottle. The mixture then heated and stirred simultaneously in a media bottle. In the nutrient agar preparation process, aseptic practices were maintained in order to prevent cross contamination from the surrounding. The fully dissolved mixture was autoclaved at 121℃ for 15 minutes. The autoclaved agar mixture then was allowed to cool and poured into petri dishes and leave to solidify in sterilized condition. The plates then were kept in the refrigerator and preserved at 2-8℃. The plates were used to spread the water samples.

b) Xylose Lysine Deoxycholate (XLD) Agar

The agar mixture, 27.5 g of XLD powder were suspended into 800ml of distilled water then filled until 1L with distilled water. The mixture then was heated and stirred simultaneously. In the XLD agar preparation process, aseptic practices were maintained in order to prevent cross contamination from the surrounding. The fully dissolved mixture was put in water bath at 100℃ for 15 minutes. The agar mixture was allowed to cool and poured into petri dishes and was left to solidify in sterilized condition. The plates then were kept in the refrigerator and preserved at 2-8℃. The plates were used to spread the water samples.

c) MacConkey Agar

The agar mixture was prepared, 25.77 g of nutrient agar powder suspended into 800ml of distilled water then filled until 1L with distilled water. The mixture then was heated and stirred simultaneously in a media bottle. In the MacConkey agar preparation process, aseptic practices were maintained to prevent cross contamination from the surrounding. The fully dissolved mixture is required to be autoclaved at 121℃ for 15 minutes. The autoclaved agar mixture allowed to cool and poured into petri dishes and leave to solidify in sterilized condition. The plates then were kept in the refrigerator and preserved at 2-8℃. The plates were used to spread the water samples.

d) Violet Red Bile (VRB) Agar

The agar mixture was prepared by 27.5 g of VRB powder was suspended into 800ml of distilled water then filled until 1L with distilled water. The mixture was shake vigorously. In the VRB agar preparation process, aseptic practices were maintained to prevent cross contamination from the surrounding. The fully dissolved mixture then was put in water bath at 100℃ for 15 minutes. The agar mixture was allowed to cool and poured into petri dishes and leave to solidify in sterilized condition. The plates then be kept in the refrigerator and preserved at 2-8℃. The plates were used to spread the water samples.

e) Triple Sugar Iron (TSI) Slant

The agar mixture was prepared by 32.21g of TSI agar powder was mixed into 800ml of distilled water then filled until 1L with distilled water. The mixture then heated and stirred simultaneously in a beaker. In the TSI agar preparation process, aseptic practices were maintained to prevent cross contamination from the surrounding. The fully dissolved mixture was autoclaved at 121℃ for 15 minutes. The autoclaved agar mixture then allowed to cool and poured into test tube. The agar then set in the slopped form to produce slant and capped and leave to solidify in sterilized condition. The TSI then were examine immediately after it was ready to be use.

3.3.3 Sample preparation

50 mL of sample were used within 3 hours of collection kept in sterilize falcon tube. Then each point of sample collection was recorded as well as its coordinate, time and date labelled on the falcon tube. Serial dilutions were carried out using sterile distilled water up to 10^{-5}.

3.3.4 Total Plate Count

Total Plate Count (TPC) were used to calculate the total number of bacteria in the selective growth media (Arifan et al., 2019) An amount of 0.1 mL of each dilution from section 3.3.3 was spread onto Nutrient agar by using a sterile hockey stick before those plates were incubated at 37 ℃ for 24 hours inside the incubator machine.

a. Counting colonies

The colonies grown on Nutrient agar after incubated for 24 hours then were counted using colony counter (Funke Gerber). The results were recorded according to the FDA Guidelines for Aerobic Colony Counting (FDA, 2001):

i. Normal plates (30-300): The spreader-free plate was selected to calculate colony forming units (cfu). Cfu including pinpoint size on selected plate. The dilution used was recorded and total number of colonies counted were counted.

ii. Plates with more than 300 colonies: The number cfu per plate more than 300 for all dilutions was recorded as too numerous to count (TNTC).

iii. Spreaders:

    a. There are three different types of spreading colonies including: 1) a chain of colonies with not too separated obviously caused by disintegration of a

bacterial clump. 2) A water film formed between agar and bottom of plate and 3) A water film that forms on the edge or surface of agar.

b. The plates prepared from sample consist extra spreader growth including (a) Area covered by spreaders include total area inhibiting growth, more than 50 % of plate area or (b) Area of inhibit growth more than 25% of plate area are reported as spreader.

c. When required to count the plate with contain spreaders not eliminated by (a) or (b), each of three different spreader types was counted as one source. For type 1 spreader, if contain only one chain counted as a single colony. One or more chains appear from separate sources, calculate each source as one colony. Each individual growth in the chain does not count as a separate colony. Types 2 and 3 result in different colonies and were counted. The spreader count was combined with the colony count to calculate the total plate count.

iv. Plates with no CFU: The plates from all dilutions no colonies were reported as total plate count less than 1 times relevant lowest dilution used. The calculated total plate count was marked with asterisk to represent it was estimated from counts outside the range 30-300 per plate. The plate from a sample was known to be contaminated or dissatisfied, the result was recorded as laboratory accident (LA).

Controls were used for comparison of the bacterial growth on the spread samples.

b. Total Plate Count, cfu/mL

The highest dilution that contains between 30-300 cfu on Nutrient Agar was choose and multiplied by dilution factor, then divided by 0.1 mL dilution volume to get the cfu/mL. At the below show the formula cfu/mL:

Controls were used for comparison of the bacterial growth on the spreaded samples.

Formula CFU/mL

$$\textbf{CFU/mL} = \frac{\text{Number of colonies x Dilution Factor}}{\text{Volume of culture plate}}$$

3.3.5 Microbial Identification

The amount 0.1 mL using pipette from a freshwater sample was dropped and spread onto selective agar medias: XLD agar, MacConkey agar and VRB agar. All of the plates were incubated at 37 ℃ for 24 hours. The results were recorded based on the morphological characteristics of bacteria growth on the selective media. The yellow colonies on XLD agar indicate the presence of *Salmonella* typhi. The pink colonies on MacConkey agar indicate the presence of *E. coli*. The pink colonies on VRB agar indicate the presence of *coliform.*

3.3.6 Gram Staining

After the incubation period of 24 hours, a smear of bacteria sample was prepared on a clean glass slide. Firstly, crystal violet was dropped onto the fixed sample using plastic dropper and rinsed after 1 minutes with distilled water for 2 seconds. Secondly, a drop of iodine was the drop on the sample and rinsed after 1 minute with distilled water for 2 seconds. Then acetone was used to decolorise and left 30 seconds before rinsed with distilled water for 2 seconds. Final step was a drop of safranin were added and rinsed with distilled water again for 2 seconds after being left for approximately for 1 minute. The slide then was observed under microscope (Leica) under 100 x magnification with immersion oil applied to the slide. All steps were repeated for each sample grown in selective media of XLD agar, MacConkey agar and VRB agar. The results were recorded based on the characteristics of gram-positive and gram-negative bacteria.

3.3.7 Biochemical Test

a. Catalase Test

A smear of bacteria colonies was scoop out form selective media with a wire loop sterilized using a Bunsen burner were transfer onto a clean glass slide. This were done inside the lamina floor to avoid contamination. A drop of H_2O_2 then were dropped onto the slide with bacterial. The results were recorded on the observation of rapid elaboration of oxygen bubbles which indicate the presence or the absence of catalase enzyme in the bacterial colonies.

b. Triple Sugar Iron Test (TSI) Test

The top of a well-isolated colony was touched to obtain the sample needed from the selective growth media used. Then the centre of the TSI slant were stabbed to the bottom of the tube and then the surface of the agar slant was streak. The cap was set on loosely when the tube was incubated at 37°C for 24 hours inside the incubator. The results of this test were recorded according to following criteria:

i. No changes on slant which indicates the organism is not capable in fermenting glucose
ii. Bubbles, cracks or displacement of the medium which indicates gas production by the bacteria due to glucose fermentation.
iii. Yellow slant which indicates acid reaction due to high reduction of acid from glucose.
iv. Red slant which indicates alkaline reaction due to low production of acid from glucose.
v. Blackening of the medium which indicates hydrogen sulfide, H_2S production.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Determination of Total Plate Count of Each Water Sample

Firstly, the number of colonies grown on Nutrient agar of water sample were isolated for calculation. Only plates with the number of colonies between 30-300 cfu was selected for the calculation.

4.1.1 Number of Colonies on Nutrient Agar from Water Sample

Table 4.1 shows colonies of isolated from water samples grown on Nutrient agar from Hill water sample. Greyish white colour on nutrient agar where the colonies growth was observed. The plate with colonies number exceeds 300 was recorded as to Numerous to Count (TNTC). Hill water V sample with dilution of 10^{-1} have the lowest number of colonies grown on the nutrient agar with 7colonies. Hill water V sample also showed free growth of colony at dilution of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. At dilution 10^{-2}, Hill water III with 21 colonies grown have the lowest number of colonies followed by Hill water II sample with 29 colonies. At dilution 10^{-3}, Hill water II have the lowest colonies with 9, followed by Hill water IV with 13 colonies and Hill water I with 16 colonies. At 10^{-4}, Hill water II have the lowest number of colonies grown with 5, followed by Springs water I with 6 colonies and there is no growth in Hill water III and Hill water IV. All the Hill water sample does not have grown colonies at dilution 10^{-5}.

Table 4.1: Colonies on Nutrient Agar Isolated from Hill Water Sample

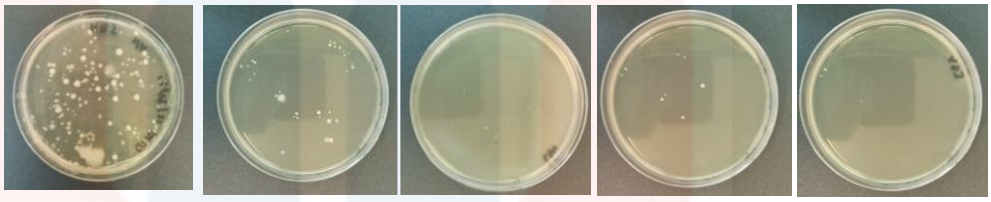
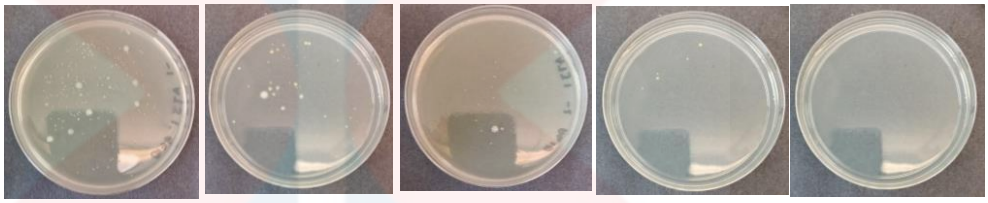
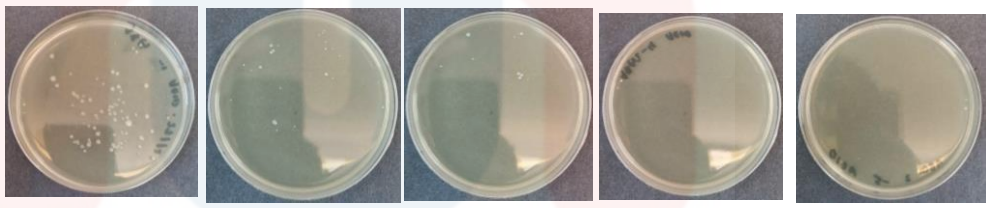
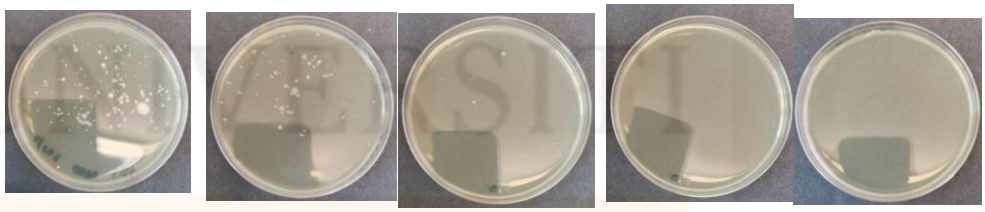
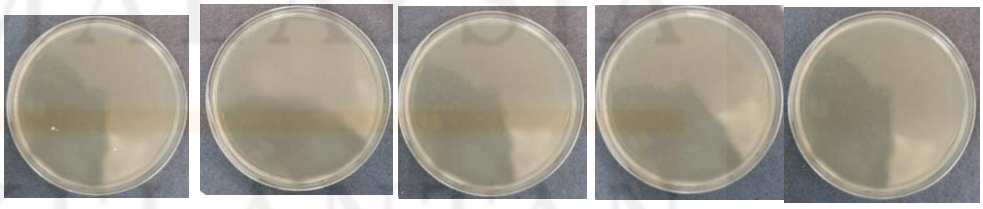
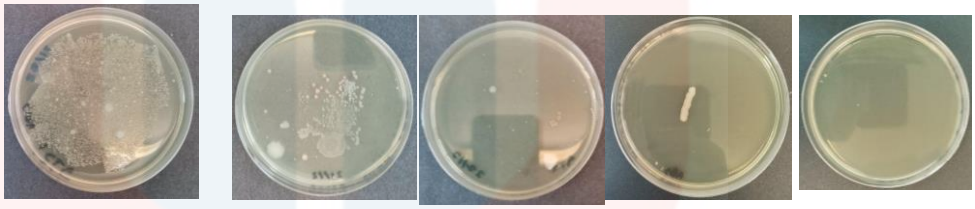
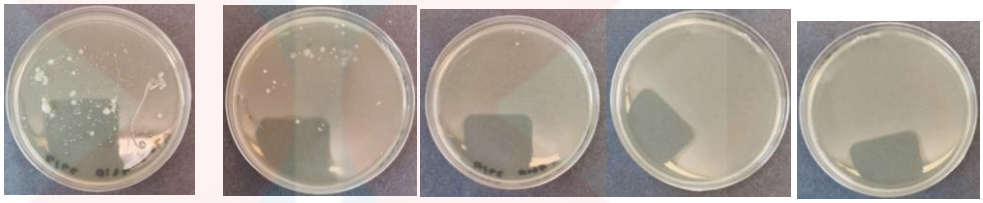
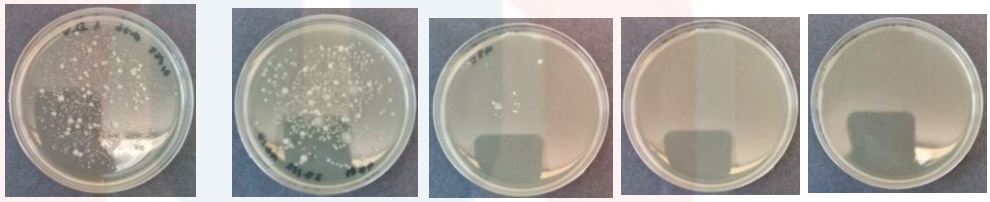
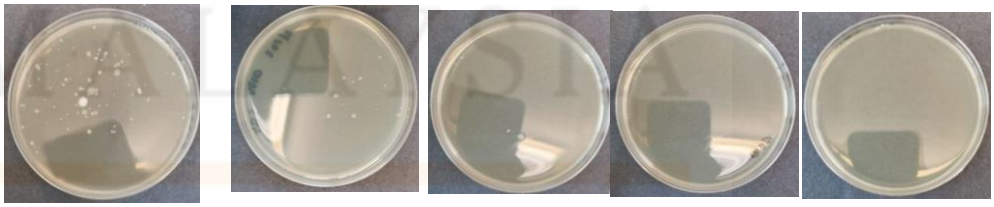
	Dilution				
Sample	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Spring Water I					
No. colony	96/Spreader	34	16	6	-
Spring Water II					
No. colony	114	29	18	5	-
Spring Water III					
No. colony	62	21	9	-	-
Spring Water IV					
No. colony	127	63	13	-	-
Spring Water V					
No. colony	7	-	-	-	-

Table 4.2 shows colonies of isolated from water samples grown on Nutrient agar from Ground Water sample. Greyish-white colour on nutrient agar where the colonies growth was observed. The plate with colonies number exceeds 300 was recorded as To Numerous To Count (TNTC). Ground water IV sample with dilution of 10^{-1} have the lowest number of colonies grown on the nutrient agar with 39 colonies. At dilution 10^{-2}, Ground water IV sample have the lowest number of colonies grown with 6 colonies, followed with Ground water V sample with 17 colonies. At dilution 10^{-3}, Ground water V sample have the lowest colonies with 3, followed by Ground water II sample with 5, while Ground water I sample have 19 colonies and Ground water III with 25 colonies also there is no growth in Ground water IV. At 10^{-4}, only Ground water I sample have colonies grown with 2 and there were no colonies grown on other Ground water sample. All of the Ground water sample does not have grown colonies at dilution 10^{-5}.

Table 4.2: Colonies on Nutrient Agar Isolated from ground water sample

	Dilution				
Sample	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Ground Water I					
No. colony	TNTC/spreader	148/spreader	19	2/spreader	-
Ground Water II					
No. colony	113/spreader	32	5	-	-
Ground Water III					
No. colony	TNTC	244	23	-	-
Ground Water IV					
No. colony	39/spreader	6	-	-	-
Ground Water V					
No. colony	78	17	2	-	-

4.1.2 Total Plate Count (cfu/mL)

Table 4.3 below showing the bacterial count of Hill water sample and Ground water sample.

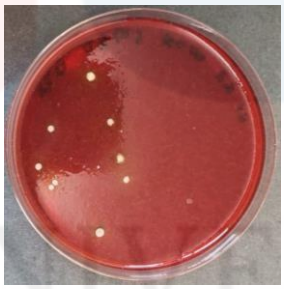
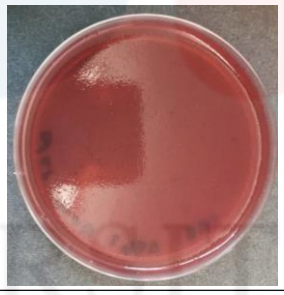
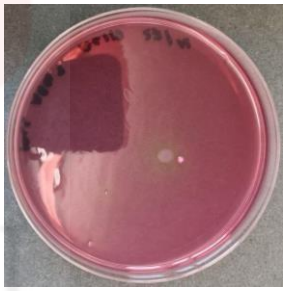
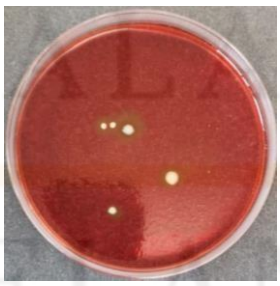
Table 4.3: Bacterial count on Hill water sample and Ground water sample

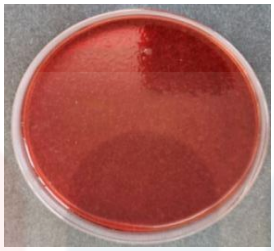
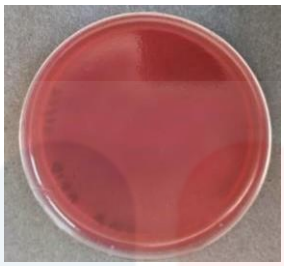
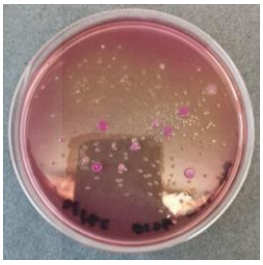
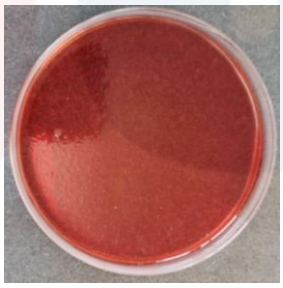
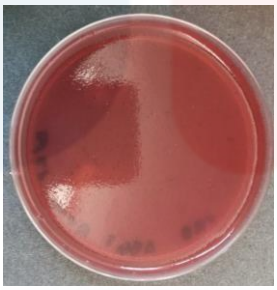
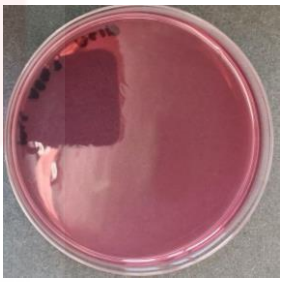
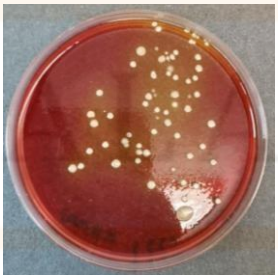
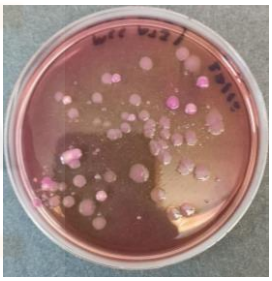
Sample	Colony forming unit, cfu/mL
Hill water I	9.6×10^3
Hill water II	1.14×10^3
Hill water III	6.2×10^3
Hill water IV	1.27×10^3
Hill water V	0
Ground Water I	1.48×10^4
Ground Water II	1.13×10^3
Ground Water III	2.44×10^4
Ground Water IV	3.9×10^3
Ground Water V	7.8×10^3

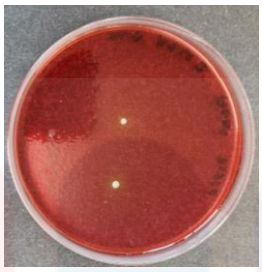
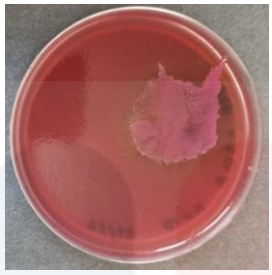
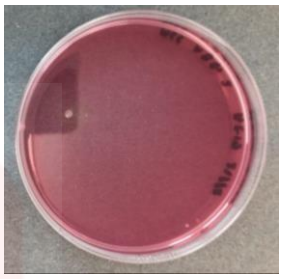
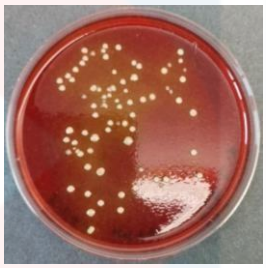
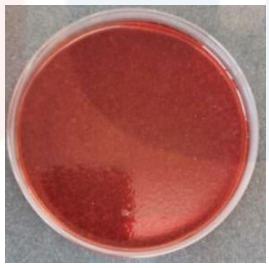
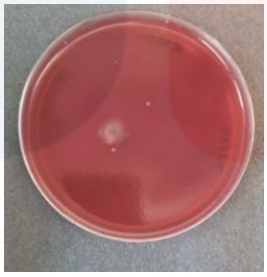
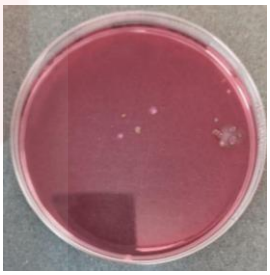
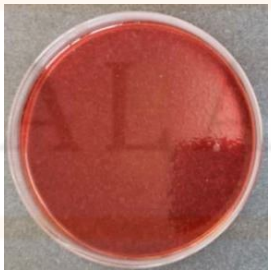
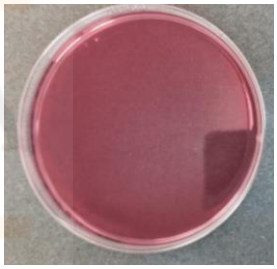
The TPC which exceed $x10^4$ shows the microbial growth in water sample is contaminated and unsafe to be consumed directly. Improper of water storage management could be the reason of why there was a high or source might be the factor of the contamination. Selective media of XLD agar, MacConkey agar and VRB agar was used to isolate pathogen bacterial from water sample.

4.2 Isolation Bacterial Grown on Selective Media from Water Sample

Table 4.4: Water Sample Result with Different Isolation Bacteria

	Selective Media		
Sample	**XLD Agar**	**VRB Agar**	**MacConkey Agar**
Control			
Colony morphology	*Salmonella typhi* growth in white without or with black centre	Coliform growth in pink or purple colonies	*E. coli* growth in pink colonies
Hill water I			
Colony morphology	Yellow halo	No colony	Pink Colonies
Hill water II			
Colony morphology	Yellow Halo	Pink Colonies	Pink Colonies

Hill water III			
Colony morphology	No colony	No Colony	No Colony
Hill water IV			
Colony morphology	Yellow halo	Pink Colony	Pink Colonies
Hill water V			
Colony morphology	No colony	No colony	No colony
Ground Water I			
Colony morphology	Yellow Halo	Pink Colonies	Pink Colonies

Ground Water II			
Colony morphology	Yellow halo	Pink Colonies	Pink Colony
Ground Water III			
Colony morphology	Yellow halo	Pink Colonies	Pink Colonies
Ground Water IV			
Colony morphology	No colony	Pink Colonies	Pink colonies
Ground Water V			
Colony morphology	No colony	No colony	No colony

The colonies grown on selective agar are identified by gram staining and biochemical tests included catalase test and TSI test. Biochemical tests were done to confirm the bacteria characteristic.

4.3 Identification Bacterial Type on Selective Media from Water Sample

Identification of bacterial were done by using gram staining and biochemical test including catalase test and TSI. Each biochemical test was carried out in order to identify the isolated colonies grown on selective media from water sample.

4.3.1 List of Isolated Bacteria from Water Sample

Table 4.4 showing the list of isolated bacteria from water sample of hill water and ground water sample grown on 3 selective media which are XLD agar, VRB agar and MacConkey agar. Alphabet A to E indicates the label of hill water sample while F to J indicates the label of ground water sample. Number "1" represented present of colonies growth on XLD agar, number "2" represented of colonies growth on VRB agar and number "3" represent colonies growth on MacConkey agar. The symbol of "-"indicates there was no colony growth on each selective media. All of these labels were used for gram staining, Catalase test and TSI test.

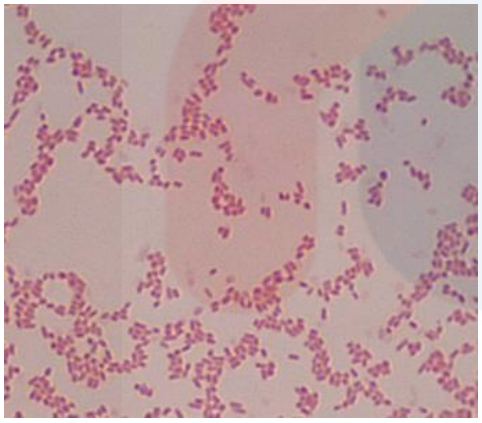
Table 4.5 Shows the List of Isolated Bacteria from Selective Media

		Selective Media		
Label	**Sample**	**XLD**	**VRB**	**MacConkey**
A	Hill water I	A1	-	A3
B	Hill water II	B1	B2	B3
C	Hill water III	-	-	-
D	Hill water IV	D1	D2	D3
E	Hill water V	-	-	-
F	Ground Water I	F1	F2	F3
G	Ground Water II	G1	G2	G3
H	Ground Water III	H1	H2	H3
I	Ground Water IV	-	I2	I3
J	Ground Water V	-	-	-

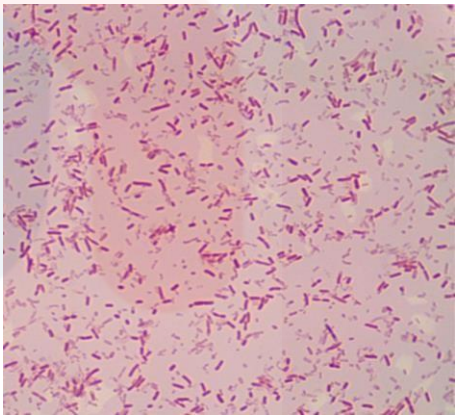
4.3.2 Gram Staining

Figure 4.1 shows the bacterial cultures under microscopic view with 100x magnification with using Gram staining method. Bacteria cultures with pink stain, rod shaped is the morphology of E. *coli*. Pink colour indicates a gram-negative bacterium as it has thin peptidoglycan layer. Based on observation made, the morphology of isolated bacteria from A3, B3, D3, F3, G3, H3, I3 from both hill water sample and ground water sample shows the same morphology of *E. coli* control and could possibly be identified as *E. coli.*

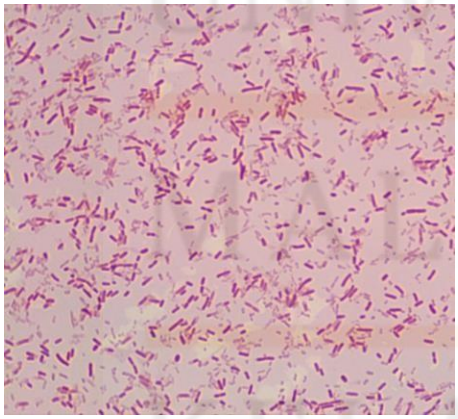
Figure 4.1: Microscopic view of bacteria cultures using Gram staining method



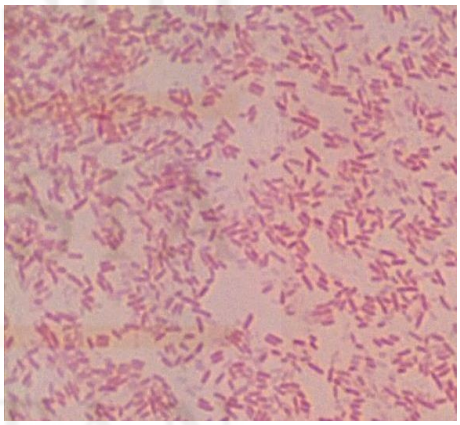
E. coli Control



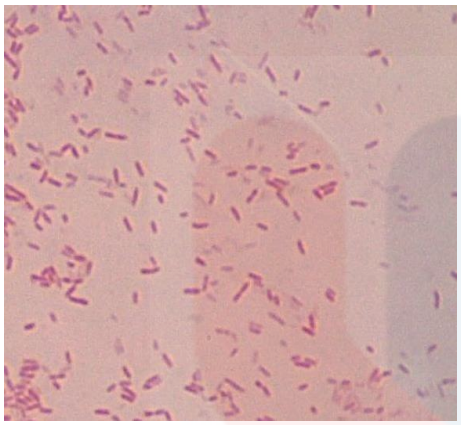
A3



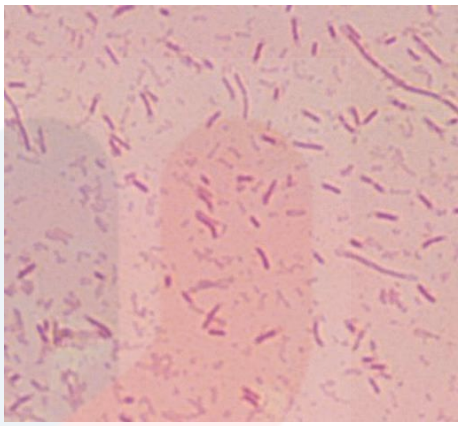
B3



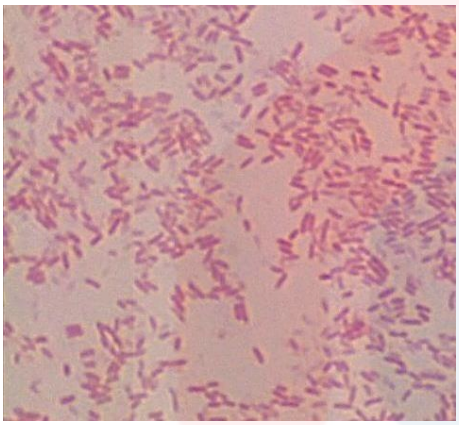
D3



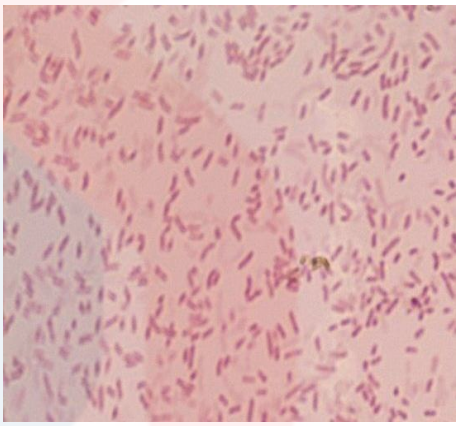
F3



G3



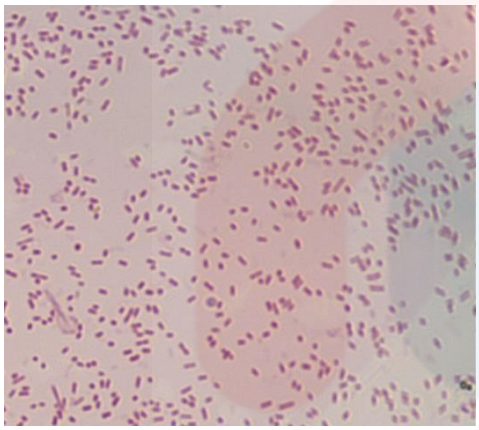
H3



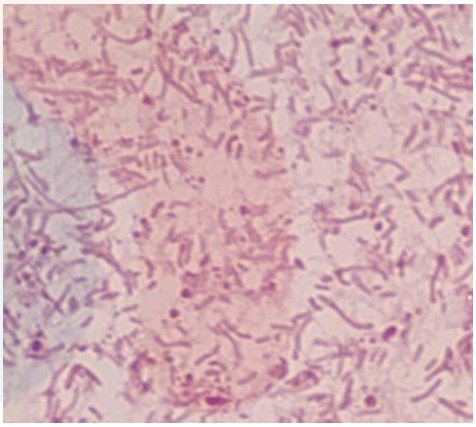
I3

Figure 4.2 shows the bacterial cultures under microscopic view with 100x magnification with using Gram staining method. Bacteria cultures with pink stain, rod shaped is the morphology of *Salmonella typhi*. Pink colour indicates a gram-negative bacterium as it has thin peptidoglycan layer. Based on observation made, the morphology of isolated bacteria from A1, B1, D1, F1, G1, H1 from both hill water sample and ground water sample shows the same morphology of *Salmonella typhi.* control and could possibly be identified as *Salmonella typhi.*

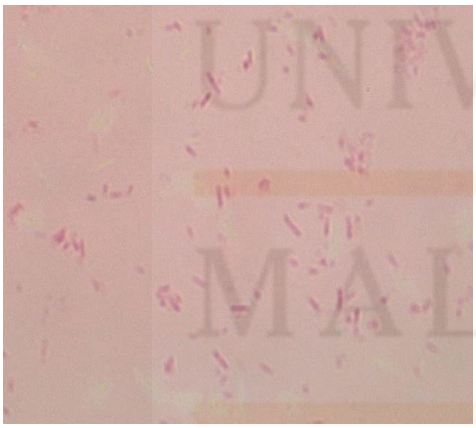
Figure 4.2: Microscopic view of bacteria cultures using Gram staining method



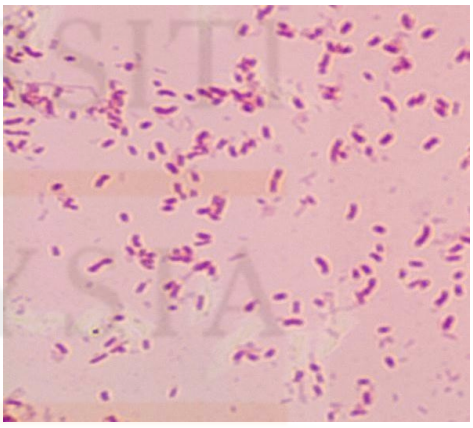
Salmonella Typhi Control



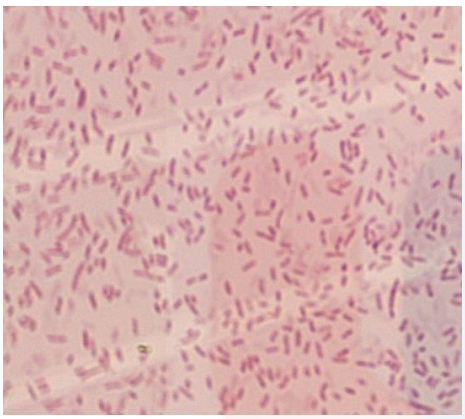
A1



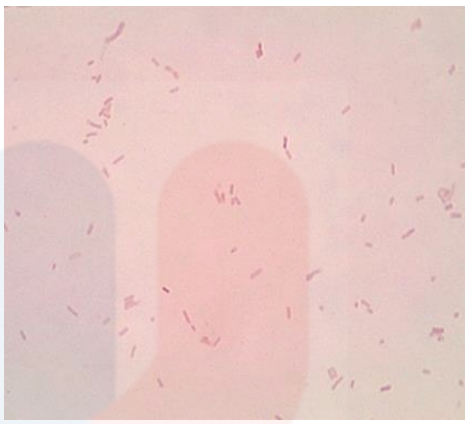
B1



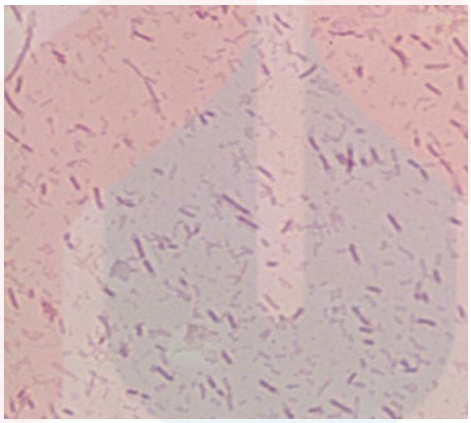
D1



F1



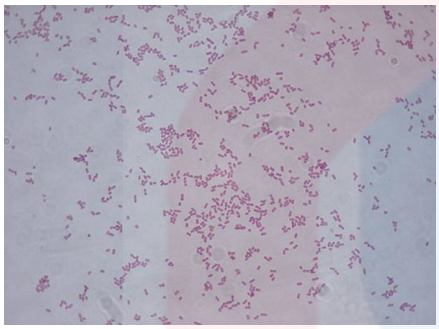
G1



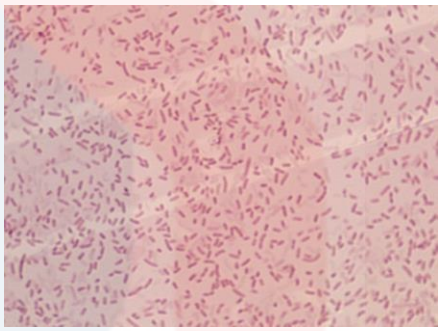
H1

Figure 4.3 shows the bacterial cultures-stained pink under microscopic view with 100x magnification with using Gram staining method. Bacteria cultures with pink stain, rod shaped is the morphology of Coliform. Pink colour indicates a gram-negative bacterium as it has thin peptidoglycan layer. Based on observation made, the morphology of isolated bacteria from B2, D2, F2, G2, H2 and I2 from both hill water sample and ground water sample shows the same morphology of Coliform control and could possibly be identified as Coliform.

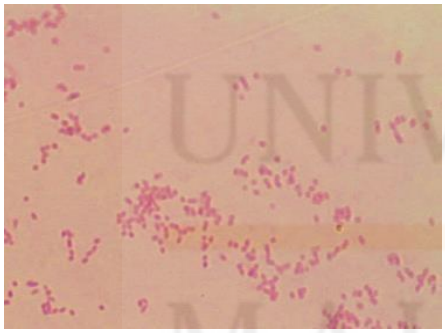
Figure 4.3: Microscopic view of bacteria cultures using Gram staining method



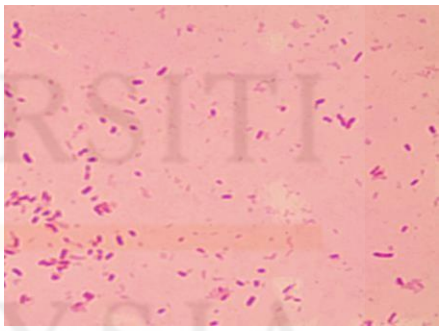
Coliform Control



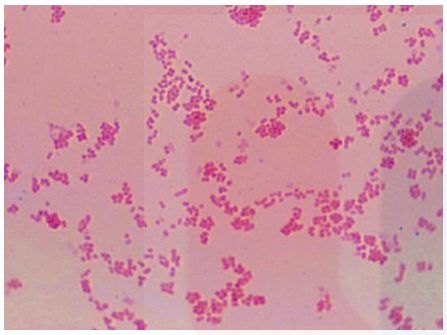
B2



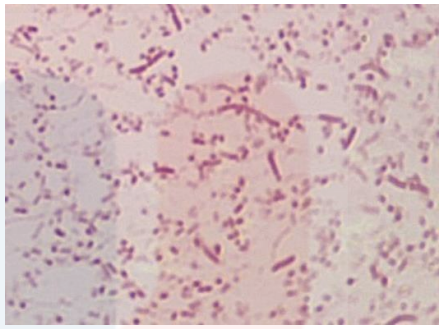
D2



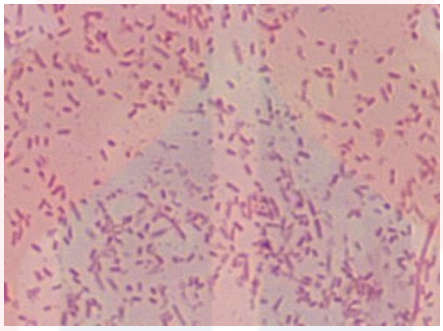
F2



H2



G2



I2

4.3.3 Biochemical test

a. Catalase test

Table 4.6 shows the catalase test undergo for colonies growth on MacConkey agar. When the bacterial colonies produce rapid elaboration of bubble indicates the presences of catalase enzyme. Based on the observation made, the isolated bacteria culture from A3, B3, D3, F3, G3, H3 and I3 was showing the same result as the control of *E. coli*.

Table 4.6: Catalase Test on Sample Isolated from MacConkey Agar

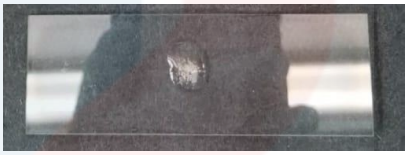
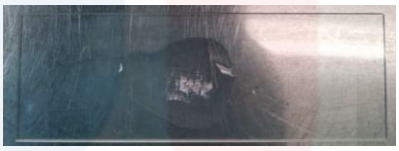
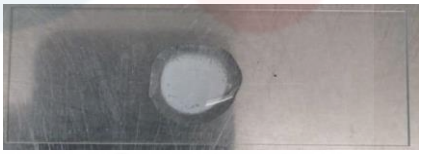
Sample	Observation
***E. coli* control**	
A3	
B3	
D3	
F3	
G3	
H3	
I3	

Table 4.7 showing the catalase test from colonies growth on XLD agar. The *Salmonella* typhi control showed rapid elaboration of oxygen bubbles. From the observation of isolated bacteria of A1, B1, D1, F1, G1, H1 have the same result of *Salmonella* typhi control.

Table 4.7: Catalase test on sample isolated from XLD agar

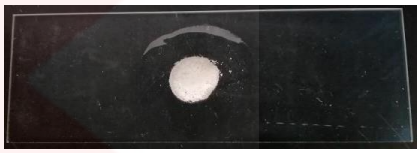
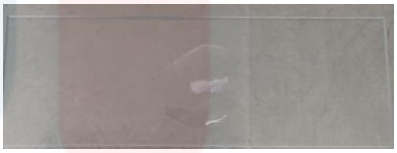
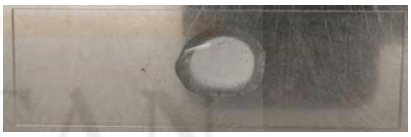
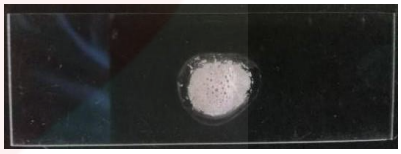
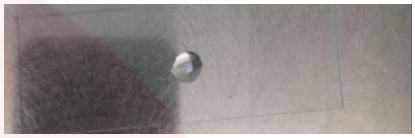
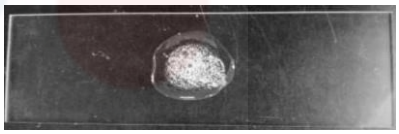
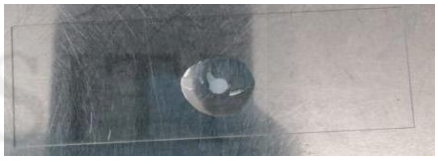
Sample	Observation
***Salmonella* Typhi control**	
A1	
B1	
D1	
F1	
G1	
H1	

Table 4.8 showing the catalase test from colonies growth on VRB agar. The Coliform control showed rapid elaboration of oxygen bubbles. From the observation of isolated bacteria of D2, F2, G2 and H2 have the same result of Coliform control. B2 and I2 does not showing bubble formation.

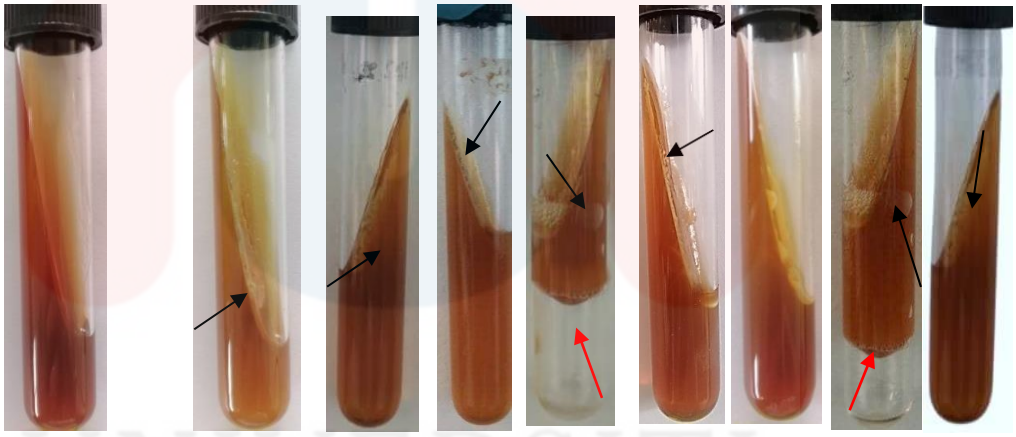
Table 4.8: Catalase Test from Colonies Growth on VRB Agar

Sample	Observation
Coliform control	
B2	
D2	
F2	
G2	
H2	
I2	

c. **TSI test**

Table 4.9 shows TSI agar slant of isolated bacteria grown on MacConkey agar. The observation was made based on uninoculated and *E. coli* control TSI agar slant. *E. coli* control TSI slant turn to yellow in colour and resulting of bubble. D3 and H3 both characteristic and displacement of agar while A3, B3, F3, G3 and I3 only bubble and colour of slant change appeared.

Table 4.9: TSI test on isolated colonies grown on MacConkey Agar

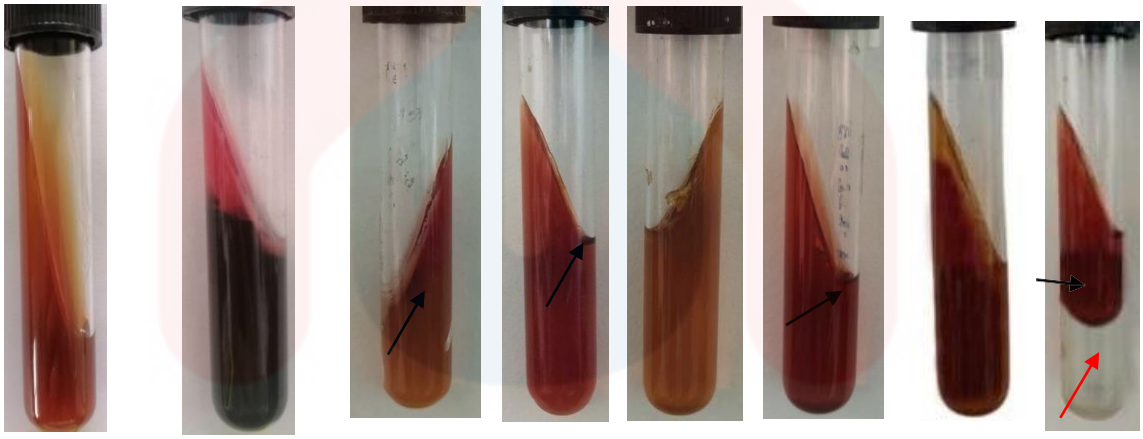
Uninoculated	*E. coli* control	A3	B3	D3	F3	G3	H3	I3
								

* → - Formation of bubble → -Displacement of agar

All of the isolated A3, B3, D3, F3, G3, H3 and I3 might be identified as *E. coli*. The displacement of agar indicates production of gas by bacterial undergoing fermentation of glucose.

Table 4.10 shows TSI agar slant of isolated bacteria grown on XLD agar. The observation was made based on uninoculated and *Salmonella typhi* control TSI agar slant. Salmonella typhi control TSI slant turn to red and blackening slant. The displacement of agar indicates production of gas by bacterial undergoing fermentation of glucose.

Table 4.10: TSI test on isolated colonies grown on XLD Agar

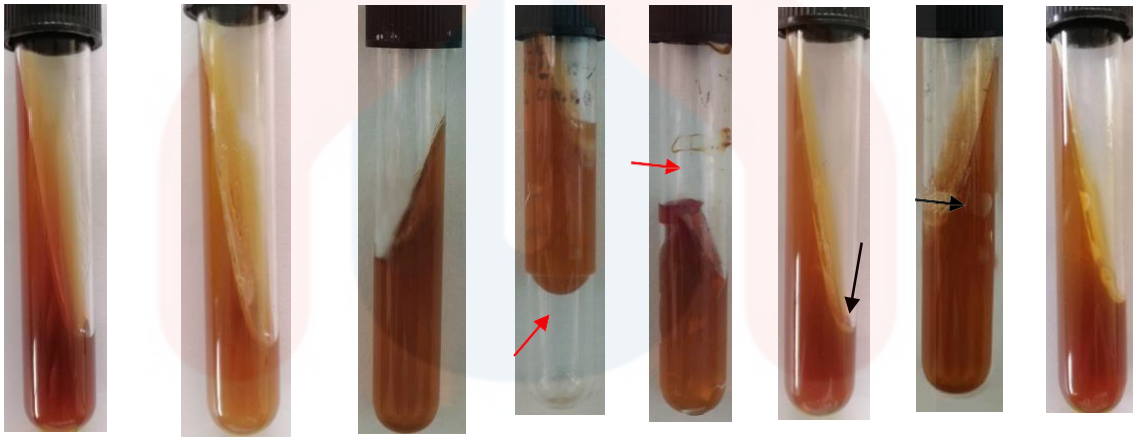
Uninoculated	*Salmonella* typhi control	A1	B1	D1	F1	G1	H1
							

*→ - Blackening of agar → - Displacement of agar

Acid reaction due to low production of acid from glucose is the reasons of the slant turn its colour to red. A1, B1, F1, G1 and H1 have the characteristic closest to *Salmonella* typhi control with some part of the turning into red colour. The displacement observed from H1 indicates the production of gas occur. There some blackening area on each sample indicates the production of hydrogen sulfide. All of the sample might be identified as *Salmonella* typhi.

Table 4.11 shows TSI agar slant of isolated bacteria grown on VRB agar. The observation was made based on uninoculated and Coliform control TSI agar slant. Coliform control TSI slant turn to yellow in colour and resulting of bubble. D2 and F2 slant turn yellow and displacement of agar. B2 and I2 only turn to yellow while G2 and H2 only bubble and colour of slant change appeared.

Table 4.11: TSI test on isolated colonies grown on VRB Agar

Uninoculated	Coliform control	B2	D2	F2	G2	H2	I2
							

* → - Formation of bubble → -Displacement of agar

The isolated D2, G2, F2 and H2 might be identified as Coliform as it shows the most characteristic as control. The change in colour of the slant to yellow indicates the acid reaction due to production of acid from glucose. Sample B2 and I2 have no gas production. The displacement of agar in sample D2 and F2 indicates production of gas by bacterial undergoing fermentation of glucose.

4.3.4 Identification of Possible Bacterial Colonies Grown on Selective Media

Through gram staining, catalase test and TSI test done on of those sample to identify all of the possible bacterial colonies grown on selective media. The identification was done through comparison based on the characteristic with the control as indicator.

a. Identification of Possible *E. coli* Colonies Grown on MacConkey Agar

Table 4.12 shows identification of possible *E. coli* colonies grown on MacConkey agar. Based on the table, isolated sample of A3, B3, D3, F3, H3 and I3 was the same as the *E. coli* control and could be the possible *E. coli*. G3 does not have rapid elaboration of oxygen bubble in catalase test and could not be identified as *E. coli*.

Table 4.12: Identification of Possible E. coli Colonies Grown on MacConkey

	***E. coli* control**	**A3**	**B3**	**D3**	**F3**	**G3**	**H3**	**I3**
Stain Colour (Gram staining)	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink
Bacteria shape (Gram staining)	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase Test	+	+	+	+	+	+	+	+
Slant Colour (TSI)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Gas production (TSI)	Present	Present	Present	Present	Present	Absent	Present	Present
Possible bacteria		*E. coli*	*E. coli*	*E. coli*	*E. coli*	-	*E. coli*	*E. coli*

- '+': Rapid elaboration of oxygen bubbles in catalase test

E. coli is a preferred indicator for freshwater recreation and its presence provides direct evidence of faecal contamination from warm-blooded animals (Water Science Scholl, 2018). The presence of E. coli in the sample may be due to contamination of the water source. The hill water might be contaminated by the faeces of animals that consume the water before it reaches the drainage of the water user. While the ground water might be contaminated in reservoirs or the surface of the well that are not covered and exposed to contamination. Thus, poor well construction can also be a source of contamination from the beginning (Wisconsin Department of Health Services, 2018).

b. Identification of Possible *Salmonella* spp. Colony on XLD Agar

Table 4.13 shows identification of possible *Salmonella* spp. Colonies grown on XLD agar. *Salmonella Typhi* control is rod-shaped with pink, a positive catalase test result, a red slant, and H_2S generation in the TSI test. The darkening of the medium shows the presence of H_2S. Based on the table, isolated sample of A1, B1, D1, F1, G1 and H1 was the same as the *Salmonella Typhi* control and could be the possible *Salmonella Typhi*. G3 does not have rapid elaboration of oxygen bubble in catalase test and could not be identified as *E. coli*.

Table 4.13: Identification of Possible *Salmonella Typhi* Colonies Grown on XLD

	***Salmonella Typhi* control**	**A1**	**B1**	**D1**	**F1**	**G1**	**H1**
Stain Colour (Gram staining)	Pink	Pink	Pink	Pink	Pink	Pink	Pink
Bacteria shape (Gram staining)	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase Test	+	+	+	+	+	+	+
Slant Colour (TSI)	Red	Red	Red	Red	Red	Yellow	Red
Gas production (TSI)	Absent	Absent	Absent	Absent	Absent	Absent	Absent
H_2S production (TSI)	Present	Present	Absent	Present	Present	Absent	Present
Possible bacteria		*Salmonella* Typhi	*Salmonella* Typhi	*Salmonella* Typhi	*Salmonella* Typhi		*Salmonella* Typhi

- ‘+’: Rapid elaboration of oxygen bubbles in catalase test

Salmonella spp. presence in water sample of ground water and hill water indicates contamination of faeces hand in processing and handling. High contamination would present in high number of *Salmonella* spp.

c. Identification of Possible Coliform Colony on VRB Agar

Table 4.14 shows identification of possible Coliform colonies grown on VRB agar. Based on the table, isolated sample of A3, B3, D3, F3, H3 and I3 was the same as the *E. coli* control and could be the possible *E. coli*. G3 does not have rapid elaboration of oxygen bubble in catalase test and could not be identified as *E. coli*.

Table 4.14: Identification of Possible Coliform Colonies Grown on VRB

	Coliform control	**B2**	**D2**	**F2**	**G2**	**H2**	**I2**
Stain Colour (Gram staining)	Pink	Pink	Pink	Pink	Pink	Pink	Pink
Bacteria shape (Gram staining)	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase Test	+	-	+	+	+	+	-
Slant Colour (TSI)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow

Gas production (TSI)	Present	Absent	Present	Present	Present	Present	Absent
Possible bacteria			Coliform	Coliform	Coliform	Coliform	

- '+': Rapid elaboration of oxygen bubbles in catalase test
- '-': No formation of oxygen bubbles in catalase test

Coliform presence in water sample indicate that the water may be contaminated by soil or faeces. The presence of Coliform also indicates that pathogen could be in the water system. Two types of coliforms might be existed in the water whether Total coliform which the contamination might come from environmental or Faecal coliform which might come from faecal contamination.

CHAPTER 5

CONCLUSION

On the whole of study, the objective was achieved whereas bacterial assessments carried on water sample of hill water and ground water were identified. Three types of bacteria acquired in this study was E. coli, Salmonella spp. and coliform through identification using gram staining and biochemical test of catalase test and TSI test. The bacterial were isolated and grown on three different selective media. MacConkey agar was used to grow E. coli, XLD agar for growing Salmonella spp. while VRB agar were used to grow coliform. All of those three bacteria were harmful to human health if detected in water source for daily consumption. The finding of the study also indicated that neither ground water nor hill water have high number of microbial counts. However, the source of contamination of the water could not be specificized as the water source use by the community of Jeli for ground water might be due to well water reservoirs that are not covered or the construction of wells that lack hygiene aspects against bacterial contamination that may contaminate the well water. This is due to lack of skills in construction of well or knowledge or lack of awareness of water quality that may be contaminated from bacteria that can be detrimental to health. At the same time, the water quality from this study is unsafe for daily usage especially for drinking and cooking without the water being treated first.

RECOMMENDATION

In term of collecting the water sample, after the water samples were collected in contaminant-free containers it must be preserved and kept cool when arriving to the lab with the temperature between 4-6℃. The cool temperature will help contaminants from breaking down. The water sample also need to be analysed minimum of 24 hours after collection because most bacteria testing in water have holding time of 30 hours only (Marianne R. Metzger,2014). Furthermore, the isolated bacterial culture on selective agar must be carried out carefully to avoid contamination to ensure a good result will be acquired. The isolated bacteria colonies also need to be analysed immediately after incubation time of 24 hours to avoid the bacterial culture from degrading due to shortage of nutrient.

REFERENCES

IOP Conference Series. (22 September, 2020). Retrieved from Earth and Environmental Science: https://iopscience.iop.org/issue/1755-1315/549/1

Arifan, F., Winarni, S., Wahyuningsih, W., Pudjihastuti, I., & Broto, R. W. (2019, October). Total Plate Count (TPC) Analysis of Processed Ginger on Tlogowungu Village. In *International Conference on Maritime and Archipelago (ICoMA 2018)* (pp. 377-379). Atlantis Press.

Awang, H., Abdullah, P. S., & Latiff, Z. A. A. (2020, August). A Preliminary Study of Local Behaviour, Perceptions and Willingness to Pay Towards Better Water Quality in Pasir Mas, Tanah Merah, and Jeli, Malaysia. In IOP Conference Series: Earth and Environmental Science (Vol. 549, No. 1, p. 012086). IOP Publishing.

Bewick, S., Parsons, R., Forsythe, T., Robinson, S., Dupon, J. (2016) Introductory Chemistry (CK12) 15.1: Structure of water. Retrieved from Libretexts websites: https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Boo%3A_Introductory_Chemistry_(CK12)/15%3A_Water/15.01%3A_Structure_of_Water

Boyd, C. E. (2015). *Water quality: An Introduction*. Springer Nature Switzerland AG

Funari, E., Manganelli, M., & Sinisi, L. (2012). Impact of climate change on waterborne diseases. Annali dell'Istituto superiore di sanita, 48, 473-487.

Hunter, P. R., Colford, J. M., LeChevallier, M. W., Binder, S., & Berger, P. S. (2001). Waterborne diseases. Emerging infectious diseases, 7(3 Suppl), 544.

IAEA. (2003). Collection and Preparation of Bottom Sediment Samples for Analysis or Radionuclides and Trace Elements.

IGOMU, E. E., ODUGBO, M., PWAJOK, D. D., BONGKO, N. L., & GOVWANG, F. P. (2017). Vom meat infusion agar medium as an alternative to nutrient agar for cultivation of Salmonella gallinarum 9R strain stock culture for fowl typhoid vaccine production. Vom Journal of Veterinary Science, 12, 11-

Jain, R. (2012). Providing safe drinking water: A challenge for Humanity. Clean Tech Environ Policy,(14),1-4. https//:doi.org//10.1007/s10098-011-04461

Khalifa, M., & Bidaisee, S. (2018). The importance of clean water. Sch J Appl Sci Res, 1(7), 17-20.

Mat, E. A. T., Shaari, J., & How, V. K. (2013). Wastewater production, treatment, and use in Malaysia. In *Safe Use of Wastewater in Agriculture 5th Regional Workshop Southeast and Eastern Asia, Bali, Indonesia*.

Sandle, T. (2016). *Pharmaceutical microbiology : Essential for quality assurance and quality control*. Woodhead Publishing. https://doi.org/10.1016/C2014-0-00532-1

Samsudin, M., Mohd Amin, M., Syed Omar, S., Yusoff, A., & Sulaiman, M. (2020). Water quality status of Pergau Reservoir Water Catchment and Lake, Jeli, Kelantan. *IOP Conference Series: Earth and Environmental Science*, 549, 012009. https//:doi.org//10.1088/1755-1315/549/1/012009

Sefie, A., Aris, A. Z., Ramli, M. F., Narany, T. S., Shamsuddin, M. K. N., Saadudin, S. B., & Zali, M. A. (2018). Hydrogeochemistry and groundwater quality assessment of the multilayered aquifer in Lower Kelantan Basin, Kelantan, Malaysia. Environmental earth sciences, 77(10), 1-15.

Smith, A. C., & Hussey, M. A. (2005). Gram stain protocols. American Society for Microbiology, 1, 14.

Wan, Z. W. M. T., Nur, H. H., Ismail, Y., Kamaruzaman, M., Johari, A. L., & Rohaimah, D. (2014). Integrated Assessment Of Groundwater Recharge In The North Kelantan River Basin Using Environmental Water Stable Isotopes, Tritium And Chloride Data.

Water treatment processes. (2021). Retrieved 29 April 2021, from https://www.hunterwater.com.au/our-water/water-supply/water-quality/how-we-protect-our-water-supply/water-treatment-processes

World Health Organization, & UNICEF. (2013). *Progress on sanitation and drinking-water*. World Health Organization.

https://microbenotes.com/catalase-test-principle-procedure-and-result-interpretation/#:~:text=The%20catalase%20test%20is%20a,catalase%20enzyme%20in%20the%20organism.&text=The%20enzyme%20neutralizes%20the%20bactericidal,the%20pathogenicity%20of%20the%20organism.