SALMONELLA, TOTAL COLIFORM, FAECAL COLIFORM, *ESCHERICHIA COLI*: INVESTIGATION OF WELL WATER IN THE VICINITY OF BADANG, KOTA BHARU

by

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree celcius
µm	Micrometer
CFU	Colony forming unit
df	Degrees of freedom
DO	Dissolved oxygen
EC	*Escherichia coli*
EMB	Eosine Methylene Blue
FC	Faecal coliform
Kg.	Kampung
MDGs	United Nations Millenium Development Goals
ml	Mililiter
MPS	Multi-probe system
MOH	Ministry of Health
n.d	No date
Sal	Salinity
SPSS	Statistical Package for the Sosial Sciences
TC	Total coliform
TDS	Total dissolved solid
Temp	Temperature
USEPA	United State Environmental Protection Agency
UV	Ultra violet
VTEC	Verotoxigenic
WHO	World Health Organization
WQA	Water Quality Association

XLD Xylose Lysine Deoxycholate

SALMONELLA, BAKTERIA KOLIFOM, KOLIFOM TINJA DAN *ESCHERICHIA COLI* : KAJIAN TERHADAP AIR TELAGA DALAM MUKIM BADANG, KOTA BHARU

ABSTRAK

Kajian telah dijalankan terhadap *Salmonella*, bakteria kolifom, kolifom tinja dan *Escherichia coli* di dalam air telaga yang diambil dari 34 titik persampelan yang terletak di tujuh kawasan dalam Mukim Badang, Kota Bharu. Kawasan yang terlibat dalam kajian ini ialah Kampung Kijang, Kampung Che Latif, Kampung Semut Api, Kampung Badang, Kampung Pulau Pisang, Kampung Pulau Kundur dan Kampung Dal. Air telaga telah diambil sebanyak dua kali pada setiap titik persampelan bermula dari Mac 2016 sehingga April 2016. Tujuan kajian ini dijalankan adalah untuk mengkaji kehadiran *Salmonella*, bakteria kolifom, kolifom tinja dan *Escherichia coli.* Selain itu, ia dijalankan untuk mencari faktor yang mempengaruhi kemampuan hidup bakteria tersebut di dalam air telaga. Ujian makmal terhadap bakteria telah dilakukan menggunakan teknik penapisan membran dan teknik cawan gores. Hasil kajian ini menunjukkan kehadiran sebahagian daripada bakteria tersebut berhubung kait dengan empat faktor iaitu pH, oksigen terlarut, kedalaman telaga dan jarak telaga dan tangki kumbahan, pada $p < 0.05$. Selain itu, jumlah bakteria tersebut didapati berbeza antara telaga tertutup, telaga separa tertutup dan telaga terbuka. Jumlah bakteria lebih tinggi dijumpai di dalam telaga tertutup. Keseluruhannya, tiada *Salmonella* dijumpai di mukim Badang. Bilangan bakteria kolifom di kesemua kawasan mukim Badang melebihi piawaian kualiti air minum yang telah dikeluarkan oleh Kementerian Kesihatan Malaysia (0 CFU/100ml).

SALMONELLA, TOTAL COLIFORM, FAECAL COLIFORM, _ESCHERICHIA COLI_: INVESTIGATION OF WELL WATER IN THE VICINITY OF BADANG, KOTA BHARU

ABSTRACT

A study of *Salmonella*, total coliform, faecal coliform and *Escherichia Coli* in well water collected from 34 sampling points from seven areas in the vicinity of Badang, Kota Bharu was conducted. The area involved were Kampung Kijang, Kampung Che Latif, Kampung Semut Api, Kampung Badang, Kampung Pulau Pisang, Kampung Pulau Kundur and Kampung Dal. Well water was collected two times at each sampling point from March 2016 to April 2016. The objective of this study was to investigate the presence of *Salmonella*, total coliform, faecal coliform and *Escherichia coli*. Furthermore it was done to find the factors contributing their survival in well water. The laboratory testing of bacteria was conducted using membrane filtration technique and streak plate technique. The result of this study showed some of those bacteria were significantly correlated to four factors which were pH, dissolved oxygen, depth of well and distance of well from sanitary tank, at $p < 0.05$. In addition, the numbers of those bacteria was different between close well, partially close well and open well. Higher number of bacteria was found in close well. Overall, no *Salmonella* was found in the vicinity of Badang. The number of coliform bacteria in all area in the vicinity of Badang exceed the drinking water quality standard given out by Ministry of Health of Malaysia (0 CFU/100ml)

CHAPTER 1

INTRODUCTION

1.1 Introduction

Water is one of the important needs for human. Human used water in their daily life for drink, food preparation, bath, and irrigation of plantation. Thus, water quality continues to be major concern among authority bodies, organisations, and researchers to ensure consumers get access to clean water.

World Health Organisation (WHO) is a well-known organisation which involved actively in providing guidelines for drinking water quality. They already published four editions of drinking water quality guidelines and the latest edition (Fourth Edition) has been published in 2011. The primary purpose of the guidelines for drinking water quality is to protect public health. The guidelines provide the recommendations from WHO in order to manage the risk from hazards that may compromise the safety of drinking water (WHO, 2011).

Water source may be obtained from river, stream, groundwater or treated water from water treatment plant. Contamination of water source in an area might cause people living in that area being exposed to several diseases related to poor water quality such as typhoid fever, diarrhea, and schistosomiasis. It also can affect human health as human consumed food obtained from water such as seafood caught from polluted area. The most popular issue in history related to the phenomenon is Minamata Disease which discovered in

Japan around 1950's. The disease was caused by consumption of seafood obtained from Minamata Bay which has been contaminated with organic mercury discharged from factory operated by Chiso Corporation.

Monitoring and maintaining the water quality is important to control outbreak of water borne disease. One of the approaches to monitor and maintain quality of water supply is through water treatment plant. Water treatment plant is a system created to treat raw water before water being supplied to consumer to ensure water is safe for daily usage and free from agents that can cause diseases. Generally, there are several processes or stages in order to treat the water which are screening, coagulation and flocculation, sedimentation, filtration, disinfection and fluoridation, and pH control.

In Malaysia, rivers, storage dams, and groundwater are the main resources for raw water. Most of the states in Malaysia are using surface water to meet the various water demands. Due to the global weather changes, the increasing demand and severe pollution of the surface water, groundwater becomes an important source for water supply. In Kelantan, groundwater plays a very important role in the public water supply system in which about 70% of the total water supply in the state is derived from groundwater, primarily in the Kota Bharu areas (Mohammed and Ghazali, 2009). However, the common perception that groundwater is a microbiologically safe source of drinking water is inaccurate and it is now apparent that a significant percentage of groundwater sources are contaminated by microorganisms derived from faeces (WHO, 2006)

E.coli is one of the faecal coliform which can affect human health. The presence of *E.coli* in groundwater can indicates that water supply is contaminated with human faeces and a sign of unsafe water supply and poor sanitation condition (Feng *et al.*, 2002). Consuming water or food contaminated with *E.coli* may cause diarrhea.

Salmonella enterica serovar Typhi (*S. Typhi*) is the bacterium responsible for typhoid fever, which affect more than 20 million people each year, resulting in over 200,000 deaths (Crump *et al.*, 2004). As *S.* Typhi is transmitted by the faecal-oral route, the typhoid fever burden falls almost exclusively in developing areas where sanitation is poor. Human are the natural host and reservoir for *S.* Typhi. *S.* Typhi can survive for days in ground water or seawater and for months in contaminated eggs and frozen oyster (Kothari *et al.*, 2008). Transmission of infection occurs by ingestion of food or water contaminated with faeces. Other established risk factors include recent contact with a typhoid patient or carrier, eating ice cream, flavoured iced drinks or food from street vendors, and raw fruit and vegetables grown in fields fertilised with sewage (Bhan *et al.*, 2005).

1.2 PROBLEM STATEMENT

The importance of water to human health and well being is encapsulated in the Human Right to Water and Sanitation, which entitles everyone to sufficient, safe, acceptable physically accessible and affordable water for personal and domestic uses (Dennis, 2003). Every individuals in this world deserved to access to the water that is safe for daily basis use. The failure to provide safe water supply means that the failure to satisfy the human right to obtain water that is safe to fulfill their daily life requirement.

The United Nations Millennium Development Goals (MDGs) target to halve the proportion of the population without sustainable access to safe drinking-water between 1990 and

2015 (United Nations Development Group, 2003). Reducing the number of population without sustainable access to safe drinking-water can also reduced the outbreak of water borne disease which is usually caused by pathogenic microorganisms. The microorganisms are unable to be seen with naked eyes, thus, it become threat to human health as it can gain access to human body whenever coming into contact with human either through ingestion or physical contact for examples during bathing and swimming.

In recent years, people were faced the major problems that are related to water quantity or quality issues. Water-borne like hepatitis, cholera, dysentery and typhoid were more common infectious diseases that affect large populations in the tropical regions (Mondal and Kar, 2013). Water borne disease caused by microorganisms may cause mild health effect as well as serious health effect. In certain occasion, the health effect caused by water borne microorganisms may caused death.

As water borne diseases caused by microorganims are capable to bring serious health effect to human, the content of such microorganism should be monitored and evaluated to prevent the outbreak of diseases which can caused human to suffer. Monitoring of faecal indicator and pathogenic bacteria in groundwater is important for assessing the risk of microbial contamination of groundwater.

1.3 OBJECTIVES

1.3.1 General Objective

To investigate the presence of *Salmonella* total coliform, faecal coliform, *Escherichia coli* in well water in Badang.

1.3.2 Specific Objectives

1) To determine the number of *Salmonella*, total coliform, faecal coliform and *E.coli* in well water from different sampling area in the vicinity of Badang.

2) To study the correlation between variables (temperature, total dissolved solid (TDS), salinity, pH and dissolved oxygen (DO), depth of well and distance between well and septic tank) and the number of *Salmonella*, total coliform, faecal coliform, *E.coli* in well water.

1.4 HYPOTHESIS

1) There is significant correlation between the number of *Salmonella*, total coliform, faecal coliform, *E.coli* and the water parameter.

1.5 SIGNIFICANCE OF STUDY

Human used water in their daily life for many purposes. Water supply that are contaminated with pollutant and bacteria is at high risk to cause health problem to human. So, it is important to identify the content in water supply to ensure there is no harmful organism or matter that can caused health problem to consumer.

This study intended to identify the presence of *Salmonella*, total coliform, faecal colliform, and *Escherichia coli* (*E.coli*) bacteria in well water in selected area in Badang. It was to ensure well water used by residents in Badang is clean and safe for domestic by comparing the result of this study with drinking water quality standard given by Ministry of Health (MOH).

This study also can help residents at those area to be more alert to bacteria contamination in well water especially high risk bacteria. The result from this study will prevail by MOH as a guidance of preliminary condition at selected area. Thus, early prevention can be applied.

CHAPTER 2

LITERATURE REVIEW

2.1 Water

Water is the most common and important chemical compound on earth. The availability of drinking water has been the most critical factor for survival throughout the development of all life. In the history of mankind, cultural centres were always founded in areas with a sufficient supply of freshwater (Szewzyk *et al.*, 2000).

Some changes on urban land use and land cover can significantly affects spatial and temporal patterns of runoff which can results in deterioration of surface water quality (Wilson and Weng, 2010). Evans *et al.* (2012) stated, the intense pressure of water resources was driven by population growth and the need for increased agricultural production. Increased human activity was associated with eutrophication of waterways and the resultant increases in diseases (Ashbolt, 2004). According to Yu *et al.*,(2013), urbanisation, agricultural intensification and deforestation were the primary anthropogenic forces that cause land use change and ecosystem degeneration. Land use conversion during urbanisation process increases impervious area and runoff, generates pollution and alters the configuration, composition and context of land uses. The changes occur were related to the ability of the land system to deposit, intercept, absorb and evaporate pollutants. Increased in population can caused limitation in the natural water supply. These phenomenon lead to development of sophisticated techniques and systems to obtain access to new water reservoirs (e.g. drilling of wells and building aqueducts) and to distribute water for irrigating and drinking (Szewzyk *et al.*, 2000). Rapid urbanisation, especially in developing countries like India, has affected the availability and quality of groundwater due to its overexploitation and improper waste disposal (Ramakrishnaiah *et al.*, 2009).

2.2 Water Quality

The quality of water that was used for specific purposes such as drinking, agricultural, industrial, recreational, and habitat can be measured by using several parameters like physical, chemical and biological (Giri and Qiu, 2016). According to WHO (2013), coliforms, *Escherichia coli* (*E.coli*), *Enterococci* and *Faecal streptococci* and *Bacteriophages* were among the indicators microorganism which were used in the assessment of drinking water quality. Table 2.1 shows the water quality guideline given out by Ministry of Health, Malaysia (MOH).

Table 2.1: Water quality guideline

Parameter	Group	Recommended Raw Water Quality	Drinking Water Quality Standards
		Acceptable Value (mg/liter(unless otherwise stated))	Maximum Acceptable Value (mg/liter (unless otherwise stated))
Total coliform	1	5000 MPN/100ml	0 in 100ml
E.coli	1	5000 MPN/100ml	0 in 100ml
Turbidity	1	1000 NTU	5 NTU
pH	1	5.5 – 9.0	6.5 – 9.0
Total Dissolved Solids	1	1500	1000
Colour	1	300 TCU	15 TCU

(Source: Ministry of Health)

Based on the guideline given by MOH, there should be no detection of total coliform and *E.coli* in drinking water.

2.3 Groundwater

Groundwater is used for domestic and industrial water supply and irrigation all over the world. Rapid growth of population and the accelerated pace of industrialisation increases the demand for fresh water (Ramakrishnaiah *et al.*, 2009). Groundwater is the water contained beneath the surface in rocks and soil that accumulates underground in aquifers. They are usually of excellent quality. Being naturally filtered in their passage through the ground, they are usually clear, colourless, and free from microbial contamination and require minimal treatment (Babiker and Mohamed, 2014).

Since earliest antiquity, humankind has obtained much of its basic requirement for good quality water from subteranean sources (Foster and Chilton, 2003). Groundwater contitutes 97 per cent of global freshwater and is an important source of drinking water in many regions of the world (Howard *et* al., 2006). Table 2.2 shows the proportion of groundwater in drinking-water supplies in selected European countries. In Malaysia, groundwater is also used as water supplies. Table 2.3 shows the number of well for water supplies in Malaysia. As Groundwater is an important source of drinking and household water worldwide, hence, the quality of groundwater is very important for preventing waterborne disease outbreaks and should be properly monitored (Lee *et* al., 2011).

Table 2.2: Proportion of Groundwater as a source of drinking-water in selected European Countries in 1999

Country	Propotion	Country	Propotion
Austria	99%	Bulgaria	60%
Denmark	98%	Finland	57%
Hungary	95%	France	56%
Switzerland	83%	Greece	50%
Portugal	80%	Sweden	49%
Slovak Republic	80%	Czech Republic	43%
Italy	80%	United Kingdom	28%
Germany	72%	Spain	21%
Netherlands	68%	Norway	13%

(Source: Howard *et al.*, 2006)

Table 2.3: Number of well for water supplies in Malaysia

State	Number of well in 2010		Number of Users
	Aluvium	Hard Rock	
Sabah	5	2	11390
Sarawak	5	0	5000
Johor	2	5	5700
Perak	0	0	2900
Melaka	1	1	920
Kelantan	6	0	900
Kedah	0	2	2500
N. Sembilan	1	1	1280
Selangor	2	0	2200
Terengganu	0	0	800
Pahang	3	0	200
Perlis	0	0	0
P.Pinang	0	0	0

(Adopted from: Natural Resource and Environment Department (NRE), 2010)

In Kelantan, treated water resources remain an issue where the coverage of treated water is less than 70%. In addition, most of Kelantan citizens receiving treated water resources also used well water supply at the same time (Utusan Online, 2015).

2.4 Groundwater Contamination

Emerging pathogens in drinking water have become increasingly important during the decade. These include newly-recognised pathogens from faecal sources such as *Cryptosporidium parvum, Campylobacter* spp., and rotavirus, as well as pathogens that are able to grow in water distribution systems, like *Legionella* spp., mycobacteria, and aeromonads (Szewzyk *et al.*, 2000). Groundwater contamination is a widespread problem in the process of urbanisation. It has been shown that developed areas show higher faecal densities than less developed areas and the major source of faecal coliforms in urban areas was the stormwater run-off (Kelsey *et al.*, 2004). Rapid development of industry, especially the development of the chemical industry has resulted in an ever-present contamination of all kinds of natural water systems (Szewzyk *et al.*, 2000). In the other hand, the most common water-quality problem in rural water supplies is bacterial contamination from septic tank, which are often used in rural areas that don't have a sewage-treatment system. Effluent from a septic tank can percolate down to the water table and maybe into a homeowner's own well.

Contamination of groundwater with pathogenic microorganisms is generally believed to result from migration or introduction of faecal material into the subsurface. Previous studies had shown that up to half of all US drinking water wells tested had evidence of faecal contamination (Macler and Merkle, 2000).

When aquifers become polluted, contamination is persistent and difficult to remediate due to their large storage, long residence times and physical inaccessibility (Foster and Chilton, 2003). Groundwater contamination is an irreversible and unnoticeable process. Effort to improve groundwater condition may have limitation due to prohibitive costs and time requirement and the quality of contaminated groundwater cannot be restored by stopping the pollutants from the source (Causape *et al.*, 2009). The vulnerability factors for groundwater contamination include wastewater flow, vadose-zone conditions (redox, percent saturation, hydraulic conductivity), depth to groundwater, groundwater conditions (redox, hydraulic conductivity, gradient), lot size, and nearest down-gradient well supply (McQuillan, 2006).

Besides that, there are several factors that could affect the probability of groundwater resources being contaminated by pathogens. The factors include the number of pathogens released to the environment initially, the prolonged existence of the pathogens in the environment, filtration of pathogens through the soil and vadose zone, and the hydrological regime (Close *et al.*, 2008). Table 2.4 shows waterborne pathogens and their significance in water supplies.

Table 2.4 Waterborne bacteria and their significance in water supplies

Pathogen	Health Significance	Persistence in water supplies
Bacteria		
Escherichia coli - Pathogenic	High	Moderate
Legionella spp.	High	May multiply
Salmonella typhi	High	Moderate
Other salmonellae	High	May multiply
Shigella spp.	High	Short
Vibrio cholera	High	Short to long

(Adopted from: World Health Organisation (WHO), 2008)

2.5 Waterborne Disease

Water near agricultural areas may contain harmful organic material from pesticide or fertiliser application. Chemicals from pesticides and fertilisers in water may increase cancer risk and reproductive problems, and can impair eye, liver, kidney, and other body functions (Water Quality Association (WQA), n.d). Adverse health outcomes are associated with ingestion of unsafe water, lack of access to water, lack of access to sanitation, contact with unsafe water, and inadequate management of water resources and systems, including in agriculture (World Health Organisation, 2002). In drinking water, microbes such as bacteria and viruses are the contaminants with the greatest chance of reaching levels high enough to cause acute health effects (WQA, n.d).

Water is essential for maintaining life on Earth. Meanwhile, water can also serve as a media for hazardous substances and pathogenic organisms, posing substantial health threats to humans through a variety of pathways (Yang *et al.*, 2012). Waterborne disease remains one of the major health concerns in the world. Diarrhoeal diseases, which are largely derived from poor water and sanitation, accounted for 1.8 million deaths in 2002 and contributed around 62 million Disability Adjusted Life Years per annum (Mathers *et al.*, 2004). Waterborne disease caused the death of millions human beings anually (Altintas *et al.*, 2015). Outbreak of waterborne disease could be identified when there are two or more individuals who are epidemiologically linked by location of exposure to water, by the time of exposure, and by characteristics of illness, and epidemiological evidence implicate water as the probable source of illness (Lee *et al.*, 2002).

Although substantial advances in biomedical sciences and public health measures have facilitated control of many infectious diseases in the past century, the world has witnessed an increasing incidence and geographical expansion of emerging and re-emerging infectious disease (Jones *et al.*, 2008).. The migration of disease causing microorganisms into groundwater might constitute a risk of disease outbreaks, especially if setback distance or water detention time between the source of contamination and abstraction point are too close at the same time is distributed without disinfection (Hanne and Liv, 2010).

Viruses, bacteria and parasites are all considered as waterborne pathogen which are infectious and may cause disease to people. Viruses are the smallest waterborne pathogen that are highly infectious. In addition, viruses can live for a long time in aqueous environment and many of them are resistant to anti-virus. For example norovirus remain infectious even after 2 months living in groundwater (Seitz *et al.*, 2011).

Bacteria are also infectious as, but they are less infectious compared to virus because they can be disinfect by chlorination. Specific type of bacteria are known as a causal for specific disease (*Shigella* caused shigellosis, *Salmonella* typhi caused typhoid fever and *Vibrio cholerae* caused cholera). Woodall (2009) stated that bacteria is known as the major agents of diarrhoea. Table 2.5 shows microorganisms and their respective major disease and sources.

Table 2.5: Microorganisms and their respective major disease and sources.

Name of microorganisms	Major disease	Major reservoirs and primary sources
Salmonella typhi	Typhoid fever	Human faeces
Enteropathogenic E.coli	Gastroenteritis	Human faeces
Vibrio cholera	Cholera	human faeces and freshwater zooplankton
Legionella pneumophila	Acute respiratory illness	Thermally enriched water
Leptospira spp.	Leptospirosis	Animal and human urine

(Adapted from: Ashbolt, 2004)

2.6 *Salmonella*

Salmonella is a gram-negative, facultative anaerobic, rod-shaped, non-sporeforming, motile bacteria which belongs to the family of Enterobacteriaceae (Franz and van Bruggen, 2008). Members of the genus *Salmonella* are divided into two species which are *Salmonella enterica and Salmonella bongori* (López *et al.*, 2011).

Salmonella species are probably the most well-known bacterial foodborne pathogen (Eelco and Ariena, 2008) and is one of the leading causes of intestinal illness all over the world as well as the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fevers and water is known to be a common vehicle for the transmission of typhoidal *Salmonella serovars* (Levantesi *et al.*, 2012). It was believed that, for every ten cases of *Salmonella typhi (S.typhi)* infection, there are one or two cases of paratyphoid fever. *Salmonella* infections may be divided into five categories which are gastroenteritis, enteric fever, bacteremia, localised infections, and the chronic carrier state, while the most serious localised manifestations outside the gastrointestinal tract include endovascular lesions, osteomyelitis, and meningitis (Soravia-Dunand *et al.*, 1999). Vascular infections due to *Salmonella* can involve the thoracic and the abdominal aorta, as well as coronary arteries, peripheral arteries, or vascular grafts and prosthetic valves (Meerkin *et al.*, 1995).

2.7 Coliform Bacteria

Coliform bacteria are a cluster of bacteria that are linked together because they can grow together as a single group (Ibfelt *et al.*, 2015). Coliform bacteria are recognized as a gram-negative, rod-shaped, nonspore forming lactose fermenter which includes *Escherichia* sp, *Klebsiella* sp, *Enterobacter* sp, and *Citrobacter* sp (Johnson *et al.*, 2005).

There are three groups of coliform bacteria which are total coliform, faecal coliform and *E.coli* and each group is an indicator of drinking water quality and has their own level of risk. Total coliform is a large collection of different kind of bacteria, while faecal coliform are types of total coliform and *E.coli* is a subgroup of faecal coliform (Washington State Department of Health, 2016).

Coliform bacteria are typically not harmful to humans, but they are monitored as indicator organisms to detect the possible presence of faecal contamination and human health risk (Smith *et al.*, 2008). The presence of coliform bacteria in water is a sign that the water has been contaminated with human or animal wastes and the water cannot be considered safe for drinking purpose (Devi *et al.*, 2008). Faecal coliform were also been considered as an indicator organism in recreation waters by United States Environmental Protection Agency (USEPA). According to Vermont Department of Health (2016), coliform bacteria in drinking or swimming water will not necessarily cause health effect. However, other disease-causing organisms may also be present as there is the presence of coliform bacteria.

2.8 *Escherichia Coli* (*E.coli*)

E.coli bacteria normally live in the intestines of people and animals. Most of *E.coli* strain are harmless and actually are an important part of a healthy human intestinal tract. However, some *E.coli* are pathogenic which can cause illness like diarrhea or illness outside of the intestinal tract (Centers for Disease Control and Prevention, n.d). *E.coli* occurs in diverse forms in nature, ranging from commensal strains to those pathogenic on human or animal host (Jan *et al.*, 2011). Commensal *E.coli* can be found in the intestine of poultry, cattle, and pigs that are used for food production, and food of animal origin can be contaminated with *E.coli* during slaughter of the animals (James *et al.*, 2005).

Most *E.coli* are harmless but a small proportion has evolved into pathogenic type which capable to cause serious clinical symptom in humans (Eelco and Ariena ,2008). Hammerum and Heuer (2009) stated, in human, the majority of infections caused by *E.*coli are not harmful (e.g. uncomplicated urinary tract infections), whereas other infections (e.g. blood stream infections) may be lethal. Harmless type of *E.coli* live in the intestinal system of mammals and assists its host in the breakdown of particular carbon compounds. On the other hand, pathogenic type of *E.coli* possess capacities that are harmful to their hosts, for example verotoxigenix (VTEC) *E.coli* (Jan *et al.*, 2011). The VTEC strains are capable of producing verotoxins (Taylor, 2008) and could lead to bloody diarrhoea, which eventually culminates in the haemolytic uraemic syndrome (Jan *et al.*, 2011).

WHO (2014) stated that, *E.coli* could be found in sewage, treated effluents and all natural waters and soils subject to recent faecal contamination, whether from humans, wild life or agricultural activity.

The development of improved testing methods for *E.coli* and the finding that some faecal coliforms were non faecal in origins have led to the trend toward the use of *E.coli* as the preferred indicator for the detection of faecal contamination (Odonkor and Ampofo, 2013). *E.coli* is the best indicators for microbial water quality as they are still present in number which numerous enough to be detected even after significant dilution (Edberg *et al.,* 2000).

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN

The samples of well water were collected from several selected houses in the vicinity of Badang, Kota Bharu. Those samples then were further tested in laboratory for microbial content. Water parameter (temperature, total dissolved solid, salinity, pH and dissolved oxygen) was examined at the site. The result from water parameter test was used to investigate their correlation with the microbial content of well water. In addition, factors (land use, depth of well, distance between well and sanitary tank and flood history) which were believed to have potential relationship with the sample was observed and recorded.

3.2 FLOWCHART OF STUDY

Several methods were used throughout this study in order to obtain enough data and information that were related to this study. Figure 3.1 shows the flowchart of this study in which summarised the overall methodology

.

Step 1: Study Location

- A visit to Department of Health of Kelantan in Kota Bharu has been made to gather information on typhoid fever cases in Kota Bharu
- Initial survey has been made in Badang and sampling points for this study have been identified.

↓

Step 2: Samples and Data Collection

- Samples were collected from five sampling point, except for 1 village, only 4 points in seven villages in the vicinity of Badang.
- Water parameters for each sampling point were tested using YSI 556.
- Checklist was used to record other supportive data such as characteristics of well, time of sampling, and weather.

↓

Step 3: Laboratory Testing of Samples

- Samples were filtered by membrane filtration technique. A vacuum pump, a filtration unit and cellulose acetate membrane 47 mm diameter were used.
- Cellulose acetate membranes were cultured onto Mac Conkey agar, Eosine Methylene Blue Agar (EMB), and Xylose Lysine Deoxycholate (XLD).
- Colonies with the following appearance (pink on Mac Conkey; metallic green on EMB; reddish on XLD) were counted and recorded.
- The count (CFU/100ml) of each bacterium was calculated.

↓

Step 4: Data Analysis

- Data obtained from in-situ analysis and laboratory testing were analyzed using Statistical Package for the Sosial Sciences (SPSS) 22 and Microsoft Excel 2010.
- Pearson correlation test were used to test the relationship between water parameters and level of total coliform in samples.

Figure 3.1: Flow chart of study

3.2.1 Study Location

Typhoid fever cases in Kelantan show an increasing trend with 36 cases were reported from January to July 2015 and the highest cases (33%) were reported in Kota Bharu region (Adnan, 2015). From a visit to Department of Health Kelantan, the information on typhoid fever cases in Kota Bharu region was obtained (refer Table 3.1). The highest typhoid fever case in Kota Bharu was recorded in the vicinity of Badang.

Table 3.1: Reported typhoid fever cases in Kota Bharu

Vicinity	Number of Cases
Badang	**14**
Kemumin	4
Ketereh	5
Kota	2
Kubang Kerian	4
Limbat	2
Panji	11
Pendek	3
Sering	2

Based on the information, a preliminary survey was conducted in Badang. Nine preidentified housing area in Badang (Kampung Kijang, Kampung Che Latif, Kampung Semut Api, Kampung Badang, Kampung Pulau Pisang, Kampung Pulau Kundur, Kmapung Dal, Kampung Banggol and Kampung Kedai Buloh) was visited and the houses that were used well water as their resources were identified. Two out of nine locations (Banggol and Kedai Buloh) were excluded because people living at the location used treated water.

3.3 Materials and Reagents

3.3.1 Water Sampling and In Situ Analysis

200ml sterelised bottle, rope, pail, alcohol swabs, ice packs and cool box were used in water sampling. For in situ analysis of water, YSI 556 was used to analyse physical water parameter. A measuring tape was used to measure the depth of well and distance between well and septic tank.

3.3.2 Agar Preparation

For agar preparation, three types of agar powder were used which are MacConkey, Eosine Methylene Blue (EMB) and Xylose Lysine Deoxycholate (XLD). Distilled water was used to form the agar mixture. Duran bottle was used to mix the agar mixture before it was autoclaved or heated on a hot plate.

3.3.3 Membrane Filtration and Microbial Analysis of Water Samples

For membrane filtration of water samples, membrane filtration unit was used. The membrane filtration unit includes 0.45μm cellulose filter paper, filtering flask, slit-sieve disc, clamp, plastic hose connection with silicone seal and vacuum pump. For microbial analysis, the three types of agar (as mentioned before) was used to detect the present of *Salmonella*, total coliform, faecal coliform and *E.coli*. Inoculating loop was used to isolate bacteria colony and streak the bacteria on agar plate.

3.4 Samples and Data Collection

Data for this study was obtained through sampling and laboratory test of well water. Well water collection was carried out in two cycles, started in early of March 2016 and ended in early of April 2016. Well water samples were collected from five different locations (except for Kampung Dal in which samples were collected from four locations) at seven villages in Badang which were identified during preliminary survey (refer Figure 3.2). Coordinates for each sampling location was tabulated in Table 3.2.

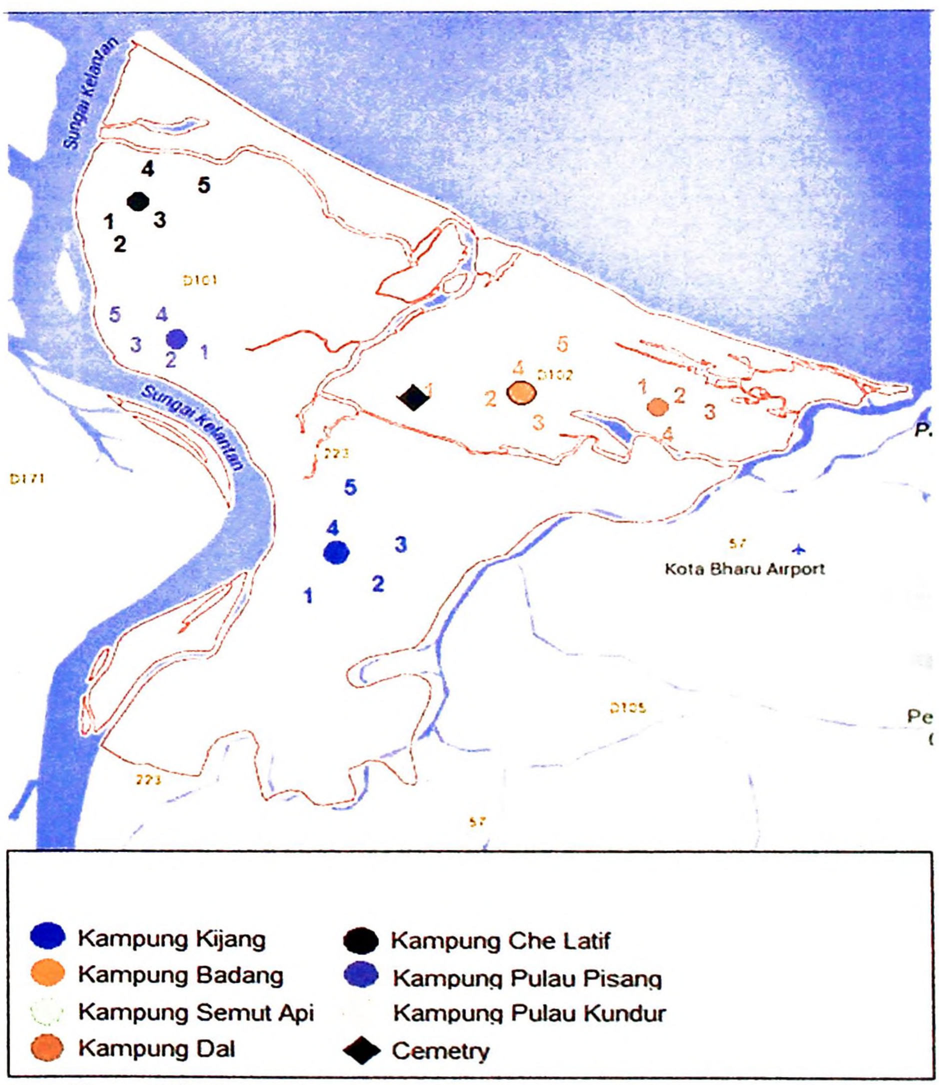

Figure 3.2: Map of the sampling area. The circles indicate villages and the numbers indicate sampling location at each village.

Table 3.2: Coordinates of sampling locations.

Sampling Location	North Longitudes	East Latitudes
Kampung Kijang 1	06°09.595'	102°15.111'
Kampung Kijang 2	06°09.523'	102°15.131'
Kampung Kijang 3	06°10.500'	102°15.207'
Kampung Kijang 4	06°11.503'	102°15.105'
Kampung Kijang 5	06°10.291'	102°15.134'
Kampung Che Latif 1	06°12.297'	102°14.937'
Kampung Che Latif 2	06°12.318'	102°14.866'
Kampung Che Latif 3	06°12.101'	102°14.114'
Kampung Che Latif 4	06°12.248'	102°14.235'
Kampung Che Latif 5	06°12.162'	102°14.438'
Kampung Semut Api 1	06°11.455'	102°16.148'
Kampung Semut Api 2	06°11.488'	102°16.166'
Kampung Semut Api 3	06°11.271'	102°16.149'
Kampung Semut Api 4	06°11.438'	102°16.134'
Kampung Semut Api 5	06°11.365'	102°16.101'
Kampung Badang 1	06°11.389'	102°15.425'
Kampung Badang 2	06°11.491'	102°15.421'
Kampung Badang 3	06°10.583'	102°16.353'
Kampung Badang 4	06°11.176'	102°16.110'
Kampung Badang 5	06°11.247'	102°16.199
Kampung Pulau Pisang 1	06°11.127'	102°14.345'
Kampung Pulau Pisang 2	06°11.128'	102°14.332'
Kampung Pulau Pisang 3	06°11.128'	102°14.244'
Kampung Pulau Pisang 4	06°11.233'	102°14.247'
Kampung Pulau Pisang 5	06°11.286'	102°14.869'
Kampung Pulau Kundur 1	06°12.359'	102°15.424'
Kampung Pulau Kundur 2	06°12.513'	102°15.325
Kampung Pulau Kundur 3	06°12.123'	102°15.241'
Kampung Pulau Kundur 4	06°12.128'	102°15.169'
Kampung Pulau Kundur 5	06°12.143'	102°15.154'
Kampung Dal 1	06°11.267'	102°16.336'
Kampung Dal 2	06°11.240'	102°16.334'
Kampung Dal 3	06°11.278'	102°16.407'
Kampung Dal 4	06°11.181'	102°16.409'

There are several factors were considered in order to identify their relationship with the presence of microbial in well water. These factors were recorded in the checklist (Appendix A). Four main factors that were observed are land use, the distance of well from sanitary tank, type of enclosure of the well (closed, partially closed, or opened), and depth of well.