UNIVERSITI PUTRA MALAYSIA

BACTERIAL INDICATORS OF PUBLIC BEACH WATER QUALITY IN MORIB BEACH, SELANGOR.

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BY

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Thesis submitted in fulfillment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

BACTERIAL INDICATORS OF PUBLIC BEACH WATER QUALITY IN MORIB BEACH, SELANGOR.

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Introduction: Beaches are one of the important coastal resources in Malaysia and become the attractions to the local and foreign tourist. Presence of infectious microorganisms in recreational water can cause human health problems such as gastrointestinal illness, health symptoms of nose, eyes, and skin. *Staphylococcus aureus* and *Salmonella* were chosen as the most suitable indicators for recreational marine waters. **Objectives:** This study was designed to determine concentrations of *Staphylococcus aureus* and *Salmonella* at Morib beach and general perceived human health symptoms from beach water exposure. It was also designed to determine the relationship between physicochemical characteristics and *Staphylococcus aureus* and *Salmonella* concentrations. **Methodology:** A cross sectional study has been conducted at Morib beach, Selangor. A set of adapted questionnaire was distributed to the visitors who exposed to beach water in Morib beach. The beach water sample was collected at eight locations in Morib beach. The water samples were filtered using membrane filtration technique. The membrane containing bacterial cells were placed on a selective medium: *Staphylococcus* 110 medium for *Staphylococcus aureus,* and xylose lysine desoxycholate agar (XLD agar) for *Salmonella.* The colonies appeared were count and analysed. Physicochemical parameters such as temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), salinity, oxidation reduction potential (ORP), and turbidity were measured on site by using YSI Profesional Plus hand-held multi probe. **Results and Discussion:** Both concentrations of *Salmonella* (87.00±40.95cfu/100ml) and *Staphylococcus aureus* (100.67±82.40 cfu/100ml) highest recorded at sampling point K16 and K17 which the possible area near to the sources of pig waste effluent runoff. The highest percentage of perceived health symptoms were none detected (56.4%). This study suggested that perceived human health symptoms among beach bathers may associated with other factors. There were significant relationship between concentrations of *Salmonella* and temperature ($r = 0.96$, $p < 0.01$), DO ($r = 0.94$, $p < 0.01$) and salinity ($r = -0.74$, $p < 0.05$). For the concentrations of *Staphylococcus aureus,* there were significant relationship between concentrations of *Staphylococcus aureus* with temperature ($r = 0.99$, $p < 0.01$), DO ($r = 0.96$, $p < 0.01$), salinity ($r = -0.80$, $p < 0.05$) and EC ($r = -0.78$, $p < 0.05$). **Conclusion:** From the finding, this study has determined the highest concentrations of *Salmonella* and *Staphylococcus aureus* were found near the pig waste effluent runoffs which were at sampling point K16 and K17. However, none of perceived health symptoms from beach water exposure was observed.

Keywords: *Staphylococcus aureus* · *Salmonella* · Gastrointestinal illness · Beach water exposure

ABSTRAK

BAKTERIA PENUNJUK KUALITI AIR PANTAI AWAM DI PANTAI MORIB, SELANGOR.

MUNASHAMIMI HAMDAN

Pengenalan: Pantai merupakan salah satu sumber pantai yang penting di Malaysia dan menjadi tarikan kepada pelancong tempatan dan asing. Kehadiran mikroorganisma berjangkit di dalam air rekreasi boleh menyebabkan masalah kesihatan manusia seperti penyakit gastrousus, gejala kesihatan hidung, mata, dan kulit. *Staphylococcus aureus* dan *Salmonella* telah dipilih sebagai petunjuk yang paling sesuai untuk perairan rekreasi marin. **Objektif:** Kajian ini telah direka untuk menentukan kepekatan *Staphylococcus aureus* dan *Salmonella* di pantai Morib dan melihat gejala kesihatan manusia daripada pendedahan air pantai. Ia juga direka untuk menentukan hubungan antara ciri-ciri fizikokimia dengan kepekatan *Staphylococcus aureus* dan *Salmonella.* **Metodologi:** Satu kajian irisan lintang telah dijalankan di Pantai Morib, Selangor. Satu set borang soal selidik telah diedarkan kepada pengunjung yang terdedah kepada air pantai di pantai Morib. Sampel air pantai diambil dari lapan lokasi di Pantai Morib. Sampel air telah ditapis menggunakan teknik penapisan membran. Membran yang mengandungi sel-sel bakteria diletakkan pada medium selektif: media *Staphylococcus* 110 untuk *Staphylococcus aureus*, dan xylose lisin desoxycholate agar (XLD agar) untuk *Salmonella*. Bilangan koloni yang kelihatan dikira dan dianalisia. Parameter fizikokimia seperti suhu, pH, oksigen terlarut (DO), kekonduksian elektrik (EC), kemasinan, pengurangan pengoksidaan (ORP), dan kekeruhan diukur dilokasi dengan menggunakan YSI Profesional Plus. **Keputusan dan Perbincangan:** Kepekatan tertinggi kedua-dua *Salmonella* ialah (87.00 ± 40.95cfu/100ml) dan *Staphylococcus aureus* ialah (100,67 ± 82,40 cfu/100ml) telah dicatat di kawasan yang dipercayai berdekatan dengan sumber larian air daripada sisa babi. Peratusan tertinggi gejala kesihatan ialah tidak dikesan iaitu sebanyak 56.4%. Terdapat hubungan yang signifikan antara kepekatan *Salmonella* dan suhu ($r = 0.96$, $p < 0.01$), DO ($r = 0.94$, $p < 0.01$) dan kemasinan ($r = -0,74$, $p < 0.05$). Untuk kepekatan *Staphylococcus aureus* terdapat hubungan yang signifikan antara kepekatan *Staphylococcus aureus* dengan suhu ($r = 0.99$, $p < 0.01$), DO ($r = 0.96$, $p < 0.01$), kemasinan ($r = -0.80$, $p < 0.05$) dan EC ($r = -0.78$, $p < 0.05$). **Kesimpulan:** Hasil ini telah menentukan kepekatan tertinggi *Salmonella* dan *Staphylococcus aureus* dijumpai dikawasan larian air daripada sisa babi. Walau bagaimanapun, tiada gejala kesihatan dilihat dari pendedahan air pantai diperhatikan.

Kata kunci: *Staphylococcus aureus* · *Salmonella* · Gastrousus· pendedahan air pantai

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LIST OF ABBREVIATIONS

CO_2:	Carbon Dioxide
DO:	Dissolved Oxygen
DOE:	The Department Of Environment
EC:	Electrical Conductivity
FIB:	Faecal Indicator Bacteria
FC:	Faecal Coliform
FS:	Faecal Streatococci
IMWQS:	Interim Marine Water Quality Standards
MWA:	Malaysian Water Association
NWQS:	National Water Quality Standards for Malaysia
ORP:	Oxidation Potential Reduction
O_2:	Oxygen
QMRA:	Quantitative Microbial Risk Assessment
RWI:	Recreational Waterborne Illness
US EPA:	United States Environmental Protection Agency
TC:	Total Coliform
WQI:	Water Quality Index
WHO:	World Health Organizations

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CHAPTER 1

INTRODUCTION

1.1 Background

Malaysia has hundreds of beautiful islands. The Star Online (2013) reported Perhentian Island in Terengganu, Juara Beach in Tioman's island, Sipadan Island in Sabah are some the making list of the world's 100 best beaches. Beaches are one of the important coastal resources in Malaysia and become the attractions to the local and foreign tourist. It promotes tourism in Malaysia as the important industry. Tourism contributes to the foreign exchange earning in Malaysia. According to Dada

et al., (2012), from year of 2006 to 2010, Malaysia has recorded an estimated RM23.6 million tourist arrivals with receipt of RM53.4 billion. It increased tourist per capita expenditure over RM2000 (EPU, 2010). Therefore, with the increased number of tourists and visitors have result in growing demand for recreational beach.

Water is required for domestic purposes, industrial purposes, agricultural purposes and recreational purposes. Beach in Malaysia is expected to provide positive impact to public accommodation. However, waste and pollution of the beaches has caused it unattractive and unsafe for the public use. A study was reported that the South China Sea is polluted with organic pollutants and solid waste. Industrial and agricultural pollution may make the situation become worse. Beach pollution and other discharges result in reduced the quality of the beach water (Sivalingam *et. al* 2007).

Water quality can be defined as the physical, chemical, biological and aesthetic properties of water. The properties of the water are influenced by dissolved or suspended constituents in water (South African Water Quality Guidelines, 1996). The characteristics of the water including temperature dissolved mineral content and number of bacteria can be measured and analyzed to determine the water quality (U.S Geological Survey, 2001). Bacteriological monitoring helps to determine the presence or absence of pathogenic microorganisms in beach water. Bacteriological monitoring of beaches is important to protect public health and promote sustainable

use of beaches. Faecal indicators are used to assess level of faecal contamination in water (Garrido-Perez *et al.,* 2008).

Water safety becomes one of the main concerns among the beach water visitors when involved in water activities such swimming, fishing, boating and others. Water quality depends on the presence or absence of pathogens such as viruses, bacteria, and protozoan that can cause disease. Pathogens can enter a water body via faecal contamination as a result of inadequately treated sewage, faulty or leaky septic systems, runoff from urban areas, boat and marina waste, combined sewer overflows, and waste from pets, farm animals, and wildlife (USEPA, 2006).

1.2 Problem statement

Utusan Malaysia (2008) reported that pig farming activities in Ladang Tumbuk, Selangor has resulted in surface and beach water pollution surrounding the area Ladang Tumbuk is located three to five km from Morib Gold Coast has released the pig farm waste direct to river. It was found that there was pig farming waste effluent runoffs into the Morib beach. Moreover, Morib beach has been modified over the years for recreational and for tourism. Most of the seafront has converted into an esplanade, leaving only a small beach at the side. Human interaction at Morib beach has brought development and services such as resorts, shops and restaurants which are convenient and useful for people. Human activities have increased the

popularity of the beach and bring more tourists. As result, anthropogenic events become one of the obvious problems at Morib beach. Initial investigation on weekend to Morib beach was found that there is surprisingly rubbish at the coastal of Morib beach.

1.3 Study Justification

Pig farm waste has polluted surrounding areas of Ladang Tumbuk including surface and beach water. However, there are no any studies have been done on pig farm waste impacted to Morib beach. Thus, preliminary study on Morib beach water quality important to ensure it is safe for public recreational activities. This study enables to detect the presence of high level of harmful microorganisms from the waste may associated with many health problems to the visitors of Morib beach. The most common illness associated with polluted surface beach water is gastroenteritis. It may cause symptoms such as nausea, vomiting, stomach ache, diarrhoea, headache and fever. Another illness related with polluted surface beach water includes ear, eye, nose and throat infections. Highly polluted beach water may cause serious illness such as typhoid fever, dysentery, cholera, and hepatitis.

Morib which located 10 km from Banting attract more than 10000 visitors especially during public holidays and festive seasons. In the study, Morib beach was chosen as the study location because it is located overlooking the Straits of Malacca

and near Ladang Tumbuk, where the waste from pig farming activities was released. Singapore Journal of International and Comparative Law (1998) stated that the Straits of Malacca and Singapore are receptacles of waste discharged from both land-based and sea-based sources. Hazardous substances and pollutant sources from agriculture activities, human settlements, industry and shipping was released into the sea. These sources of pollutant may affect the water quality of the Straits.

1.4 Conceptual framework

An analysis about the status of marine environment can be done using the assessment of the physical, chemical and biological conditions. Therefore, beach water was chosen to determine its quality status. In this study, the area of interest involved biological conditions which include microorganisms in beach water. Bacterial indicator such as *Salmonella spp.* and *Staphylococcus aureus* were chosen as the main interest. Both of bacteria can enter human body through ingestion of water and dermal contact especially through open wound. An infection caused by both bacteria may affect human health such as gastroenteritis, eye symptoms, nose symptoms, and skin symptoms.

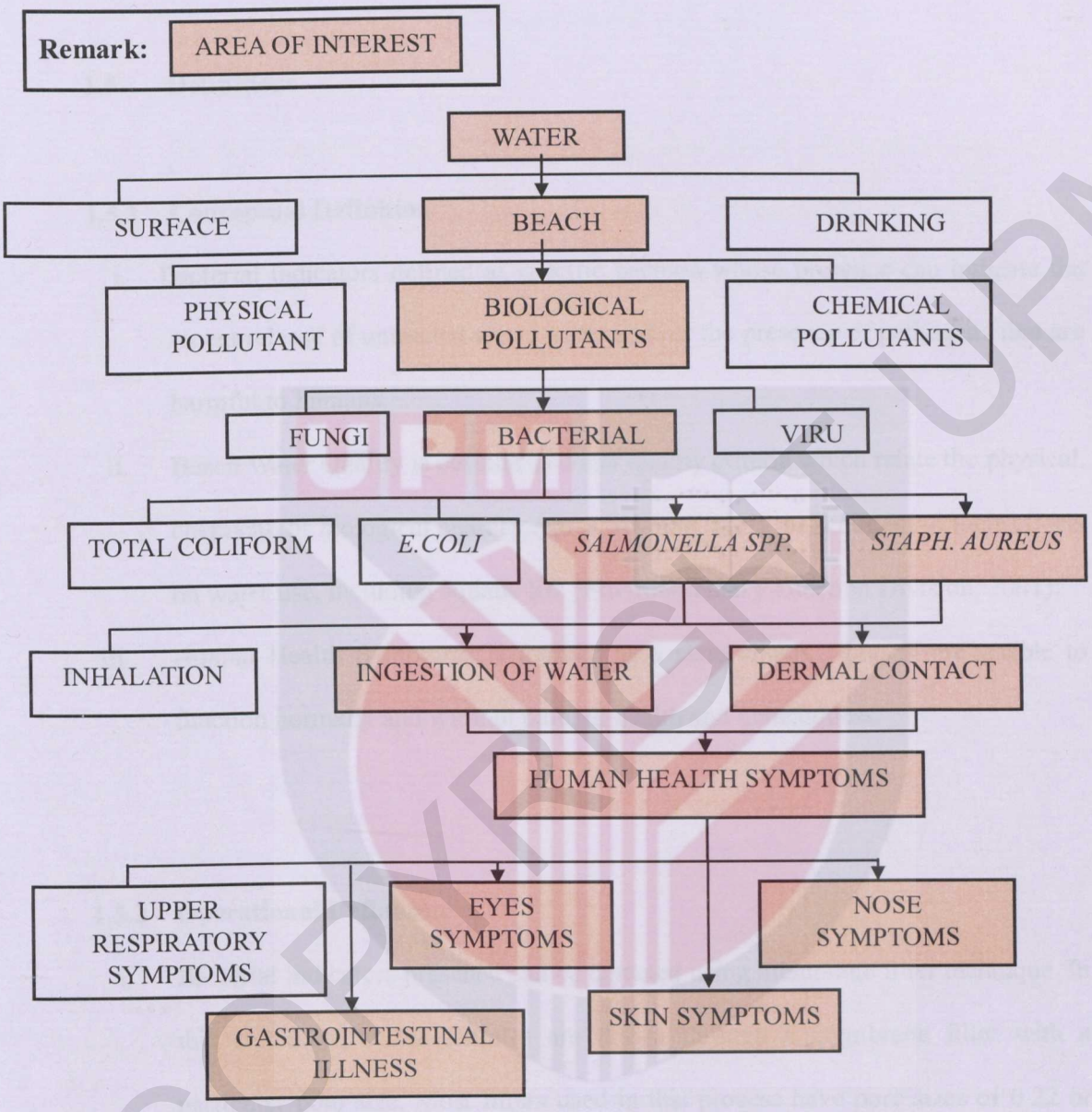

Figure 1.1: Conceptual framework of the study

1.5 Definitions

1.5.1 Conceptual Definition

i. Bacterial indicators defined as specific bacteria whose presence can indicate the recent release of untreated waste water and/ or the presence of pathogens that are harmful to humans.

ii. Beach Water Quality is defined as water quality criteria which relate the physical, chemical, or biological characteristics of water, biota, or sediment to their effects on water use, including aquatic life (Environmental Protection Division, 2001):

iii. Human Health Symptoms is defined as a state which a person are unable to function normally and without pain, ill health and unhealthiest.

1.5.2 Operational Definition

i. Bacterial indicators presence can be detected using membrane filter technique. In this technique, water samples are drawn through a membrane filter with a particular pore size. Most filters used in this process have pore sizes of 0.22 or 0.45 microns (μm). The pores allow matter smaller than 0.22 or 0.45 um to pass through the filter, whereas matter/cells that are larger get caught on top of the filter. The filter is then removed and placed on top of a growth medium. The

nutrients freely diffuse through the membrane and the microbial cells are able to grow on top of the membrane.

ii. Beach Water Quality is defined as water quality criteria which relate the physical, chemical, or biological characteristics of water, biota, or sediment to their effects on water use, including aquatic life (Environmental Protection Division, 2001):

iii. Human Health Symptoms can be determined by using questionnaire distributed to the bathers and visitor who exposed to beach water in Morib beach.

1.6 Variable

1.6.1 Independent Variables

i. Concentration of bacterial indicators (*Staphylococcus aureus* and *Salmonella spp.*) in Morib beach bathing waters.

ii. Measurement of physicochemical parameters (temperature, pH, salinity, EC, ORP, DO and turbidity)

1.6.2 Dependent Variables

i. Water quality of Morib beach

ii. Human health symptoms (gastrointestinal illness, skin symptoms, upper respiratory symptoms, eye symptoms, and nose symptoms) among visitor at Morib beach due to exposure to bathing waters.

1.7 Research objectives

1.7.1 General Objectives

To study concentrations of *Staphylococcus aureus* and *Salmonella spp.* in Morib beach and general perceived human health symptoms from beach water exposure.

1.7.2 Specific Objectives

i. To determine socio demographic of beach visitors in Morib beach.

ii. To determine and compare concentrations of *Staphylococcus aureus* and *Salmonella spp.* at Morib beach of bathing zones with other study.

iii. To determine exposure behaviour of beach visitors in Morib beach.

iv. To compare concentrations of *Staphylococcus aureus* and *Salmonella spp.* between sampling points.

v. To determine relationship between concentrations of *Staphylococcus aureus, Salmonella spp.* and physicochemical parameters.

vi. To determine commonly reported perceived health symptoms among beach visitors from bathing water exposures.

1.8 Hypothesis

There were significant relationships between the concentrations of *Staphylococcus aureus, Salmonella spp.* and physicochemical parameters.

There were significant differences between concentrations of *Staphylococcus aureus* and *Salmonella spp.* between sampling points.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbial indicators

Pathogenic microorganisms such as bacteria are microscopic single-celled organisms that act as decomposers in an estuary, breaking down plant and animal remains. Bacteria can exist on the surface water, in sediments and on the bodies of plants and animals. Other pathogenic microorganisms such as viruses and protozoan are also associated with faecal waste. These microorganisms can cause various types of diseases including typhoid fever, gastroenteritis symptoms, and cholera through ingestion of tainted water

(United States Environmental Protection Agency, 2006). They can be found in the municipal waste water discharge, storm water runoff, sewage sludge, and agricultural runoffs from livestock area. Some bacterial organisms are mobile and many congregate into colonies. In the estuary, bacteria are often found densely packed on suspended particulate matter (US EPA, 2006).

Presence of infectious microorganisms in recreational water can cause human health problems. Faecal pollution that contains pathogenic microorganisms is derived from human sewage or animal sources. Free living pathogenic microorganism exists in recreational water may cause gastrointestinal illness, health symptoms of nose, eyes, and skin. The epidemiological study in recreational stated that diarrhoea and respiratory ailments become the most common reported health problems (Wade *et al.,* 2006; Colford *et al.,* 2007; Craun *et al.,* 2005). Despite protecting public health, bacteriological monitoring of beach also helps in reducing bather's exposure to biological pathogenic bacteria, help in developing control measures and to access the suitability of current corrective measure.

The suitability of indicators for the microbiological quality of water is chosen according to specific set of criteria. The criteria needed for selecting indicators of faecal contamination in water are: (Myers *et. al.*, 2007).

- Can be easily tested
- From human or other animal origin

- High survival rate
- Is present at densities correlated with faecal contamination
- Can be used as a surrogate for many different pathogens
- Is appropriate for fresh and saline aqueous environments.

2.2 *Staphylococcus aureus* and *Salmonella spp.*

Staphylococcus is non-motile, non-spore forming and non-encapsulated Gram-positive cocci. They are able to grow aerobically and anaerobically. *Staphylococcus* usually occurs in single shape, pairs, tetrads, short chains and irregular grape-like clusters. There are three types of *Staphylococcus* species which are *Staphylococcus aureus, S. epidermidis* and *S. saprophyticus*. However, *Staphylococcus aureus* is the only coagulase-positive species and is clinically the most important (Duerden *et al.,* 1990). *Staphylococcus aureus* can be found on the anterior nasal mucosa and skin as well as in the faeces of a substantial portion of healthy individuals.

The presence of *Staphylococcus aureus* is believed to have resulted in skin rashes, wound infections, urinary tract infections, eye infections, and other infections (Calvert & Storey, 1988; Rivera & Adera, 1991). Infections of *Staphylococcus aureus* from recreational waters can only apparent until 48 hours after contact. Gastrointestinal

illness associated with *Staphylococcus aureus* is caused by a heat stable staphylococcal enterotoxin. It was believed to cause vomiting diarrhoea fever, abdominal cramps, electrolyte imbalance and loss of fluids. *Staphylococcus aureus* concentrations have been correlated to bather density and attributed to human activity (Calderon *et al.*, 1991; Charoenca and Fujioka, 1995; Papadakis *et al.*, 1997; World Health Organization, 2003). *Staphylococcus aureus* and MRSA are shed by swimmers (Robinton and Mood, 1966; Hanes and Fossa, 1970; Smith and Dufour, 1993; Elmir *et al.*, 2007; Plano *et al.,* 2011), and both are found in beach seawater and sand (Goodwin and Pobuda, 2009; Soge *et al.,* 2009; Sinigalliano *et al.,* 2010; Shah et al., 2011; Enns *et al.*, 2012).

Staphylococcus aureus can be found on the skin and mucous membrane of animals, and widely spread in the environment. It also can be detected in the gastrointestinal tract and found in sewage *Staphylococcus aureus* can be released by human contact into water environments such as swimming pools, spa pools and other recreational waters. It has also been detected in drinking-water supplies. The common route transmission of *Staphylococcus aureus* is via hand contact and direct ingestion of contaminated water (LeChevallier et al., 1980). It has been suggested that human activity is the source of *Staphylococcus aureus* at the beach (El-Shenawy, 2005). Wastewater may be another source of *Staphylococcus aureus* to the environments too.

Salmonella are gram-negative, facultative anaerobic, motile and rod shaped bacteria. *Salmonella* are comprised into more than 2000 species. They can survive in

moist environments and in the frozen state for several months. *Salmonella* bacteria can be found human and animal's intestinal tracts. *Salmonella* bacteria cause illnesses which range from asymptomatic to symptomatic infections

There has been epidemiological investigations reported that there is association of transmission of *Salmonella paratyphi*, the causative agent of paratyphoid fever, with recreational water use (Public Health Laboratory Service, 1959). *Salmonella* infections can be ranged from mild to severe. The infected person with *Salmonella* bacteria may suffer diarrhoea, headache, nausea, fever, pains in their joints and irritation of the eyes.

According to World Health Organizations (WHO) 1997, the infection associated with *Salmonella* commonly occurs through faecal-oral route, either from animal-to-human or animal-to-animal. Diarrhoea is caused by the invasion of the *Salmonella* bacteria in the terminal portion of the small intestines. This bacterium can multiply and cause inflammatory response. *Salmonella* has strong association with recreational water (Public Health Laboratory Service, 1959). It can be found in human intestines or animals including birds and reptiles.

2.3 Bacterial indicators for bathing related illnesses

Since the 1950s, health hazards associated with recreational water exposure have been studied. As result, the U.S. Environmental Protection Agency had published guidelines for ambient water quality (US EPA 1986). As result, the U.S. Environmental Protection Agency had published guidelines for ambient water quality (US EPA 1986). The guidelines recommended that for marine waters the geometric means of at least five samples taken over a 30 day period should not exceed 35 colony forming units of enterococcus per 100 mL. For fresh recreational waters, the cut-off points were 33 colony forming units (cfu) of enterococcus per 100 mL, and 126 cfu of *E. coli* per 100 mL (US EPA 1986; Wade *et al.*, 2003). These recommendations were based on associations between fecal indicator bacteria and gastrointestinal illnesses (McLellan., 2013). Bacterial indicators become the main concern because density of fecal indicator bacteria has result the occurrence of illnesses among persons with water exposure (Colford *et al.*, 2007).

The criteria for recreational water under the Beaches Environmental Assessment and Coastal Health Act of 2000 recommended that for marine waters the geometric means of at least five samples taken over a 30 day period should not exceed 35 colony forming units of enterococcus per 100 mL (US EPA 2004). For fresh recreational waters, the cut-off points were 33 colony forming units (cfu) of enterococcus per 100 mL, and 126 cfu of E. coli per 100 mL (US EPA 1986; Wade *et al.*, 2003). These

recommendations were based on associations between fecal indicator bacteria and gastrointestinal illnesses (McLellan, 2013).

Recreational waters usually contain mixture of pathogenic and non- pathogenic microorganism and free living pathogenic microorganisms. These microorganisms may come from the livestock, farming activities, domestic animals and sewage effluents. These pathogenic microorganisms may cause human illnesses such as gastrointestinal illness, upper respiratory tract, ears symptoms, eyes symptoms, and skin symptoms. Recreational waters are of particular concern because swimmers can come into direct contact with contaminated water. Polluted recreational water with faecal bacteria usually associated with gastrointestinal illness. The ingestion of water may cause gastrointestinal illness while the immersion or direct contact of contaminated water with wounds can cause an infection (Kanki and Grimes *et al.*, 2013). Gastrointestinal illness, however, may not be the only adverse health outcome associated with exposure to recreational waters. Skin, respiratory, ear, and other ailments are potentially associated with exposure to contaminated waters (World Health Organization, 2001).

High numbers of individual affected through exposure to recreational waters. Usually, individuals who acquire Recreational Waterborne Illness (RWI) do not seek medical attention because most of the illnesses tend to be mild and self-limiting. In addition, most RWIs are not reportable, so incidence levels are highly uncertain. The estimates mentioned previously were obtained using a variety of assumptions about the

contamination of water and exposures. Epidemiology studies and Quantitative Microbial Risk Assessments (QMRA) are two scientifically rigorous methods that are used to estimate rates of RWI as a function of water quality.

2.4 Human health risk

2.4.1 Gastrointestinal Illness

A gastrointestinal illness is an infection that affects the gastrointestinal system such as stomach, small intestine, and large intestine. Gastrointestinal illnesses are caused by bacteria or viruses. There are some symptoms caused by gastrointestinal illness such as nausea, vomiting, and diarrhoea. Occasionally, fever, abdominal cramps, and headache or body aches may also occur. Symptoms will vary in severity depending on the specific infection. Illnesses last for a variety of time, depending on the causative agent. Some gastrointestinal illnesses last as few as 24 hours, while others may last for several days (Virginia Department of Health, 2011).

In late 1940s and early 1950s, the U.S. Public Health Service was conducted a series of epidemiological studies at bathing places in Chicago, Kentucky, and Long Island, New York to determine the health effects associated with swimming. These studies showed positive result when was a detectable human health symptoms. Other

studies have reported skin symptoms among swimmers, while another study stated that there is significant relationship between microbial water quality and skin symptoms (Ferley *et al.,* 1989; Cheung *et al.*, 1990; Marino *et al.*, 1995). The swimmers with exposed wounds or cut have high potential to be infected by microbial water indicators.

2.4.2 Eye Symptoms

Eye symptoms are defined as any reported cases of eye redness, crusting, itching, or draining and having a skin rash. Increased rates of eye symptoms have been reported among swimmers, and evidence suggests that swimming, regardless of water quality, compromises the eye's immune defences, leading to increased symptom reporting in marine waters. Despite biological plausibility, no credible evidence for increased rates of eye ailments associated with water pollution is available (Prüss *et al.*, 1998).

2.4.3 Skin Symptoms

Skin symptoms may include tingling, burning, or itching of the skin, small reddish pimples and small blisters. There were some studies reported the elevated number of cases of skin symptoms among swimmers and association between skin symptoms and microbial water quality (Ferley *et al.,* 1989, Cheung *et al.,* 1991). The swimmer may develop symptoms within minutes or days after exposed to contaminated beach water.

The greater the number of exposures to contaminated water, the more intense and immediate symptoms of swimmer's itch will be. Controlled studies, however, have not found such association and the relationship between faecal pollution and skin symptoms remains unclear. Swimmers with exposed wounds or cuts may be at risk of infection. However, there is no evidence to relate this to faecal contamination.

2.4.4 Nose Symptom

Staphylococcus aureus mostly located in nose. Infections in nose can be localized at distant sites. *Staphylococcus aureus* most commonly causes skin infections such as boils and impetigo but it also sometimes causes serious infections like septicaemia and pneumonia. Exposure to polluted beach water may cause the infections become severe. Based on the epidemiological studies done by Stevenson (1953), it revealed that the swimmers who bathed in polluted water experience a higher incidence of upper respiratory and gastrointestinal illness.

2.5 Interaction between bacterial indicators with physicochemical parameters

Physicochemical parameters such as temperature, turbidity, Ph, salinity, dissolved oxygen (DO), electrical conductivity, and oxidation reduction potential (ORP) are used for evaluation of water quality. Each of the parameters plays important impact in the water quality.

2.5.1 Temperature

Temperature is the most important physical factors that may affect the distribution of life in oceans. Water temperature involved in the organism metabolize or breakdown and plays important role in controlling the concentrations of the bacterial indicators. Temperature of the water may also affect the exchange of the gases such as oxygen (O_2) and carbon dioxide (CO_2). Generally, the range temperature of the oceans is from 0 to 30°C (32 to 86°F). This caused by the salt content in the water lowers the freezing point of the pure water. In seawater, within the range of the temperature is between 5 to 30 °C. The slower rates of inactivation were shown at lower temperature for much kind of bacteria such as *Salmonella* spp. (Rhodes and Kator, 1988; Evison *et al.*, 1988). High temperature may cause the elevation of bacterial inactivation in seawater.

2.5.2 Turbidity

Turbidity is defined as the measure of water clarity on how much material suspended in water decreases the passage of light through the water. The examples of suspended materials include soil particles, algae, plankton, microbes and other substances. These suspended materials from the water sample may also harbour harmful bacteria. Increased in sediment influences turbidity, which is "an optical property of water based on the amount of light reflected by suspended particles" (USEPA, 1999). Turbidity can affect the water quality because it can cause the colour of the water become milky or cloudy. High turbid and cloudy water will absorbs more sun's energy rather than clear water. It results to high water temperature.

2.5.3 pH

pH is the negative of the logarithm of the hydrogen ion concentration (Ebbing, 1990). Due to low concentration of hydrogen ion, scientists have developed the pH scale to make report and interpret these numbers easier. pH scale is in logarithmic, therefore every one unit of change on pH scale, there is tenfold change in the acid or base content of a solution (Mitchell, 1997). pH level is a crucial water quality indicator because human and aquatic animals depend on water with pH level near neutral. pH is usually measured by using an electrode, which gives a digital reading. Natural water often has

pH of between 5 and 9. Water with low pH increases the solubility of nutrients like phosphates and nitrates.

2.5.4 Salinity

Salinity is a measure of the amount of salts dissolved in water sample. Salinity becomes the important physicochemical parameters in beach water quality monitoring. Salinity is measured in parts per thousand (ppt or ‰). A study from Lee *et al.,* (2010) stated that *Salmonella* poses differential survival response in relation to salinity. It has been reported that showed very low survival in sea water.

2.5.5 Dissolved Oxygen

Oxygen in water sample is measured in its dissolved form as Dissolved Oxygen (DO). Levels of dissolved oxygen will declines when more oxygen is consumed. As result, it may cause some sensitive animals move away or die. Temperature plays important role is controlling level of dissolved oxygen. High temperature hold less oxygen compares low temperature of water. Dissolved oxygen can be measured using DO meter that converts signals from a probe that is placed in the water into units of DO in milligrams per liter.

2.5.6 Electrical Conductivity

The electrical conductivity in water is affected by the presence of inorganic dissolved solids. Conductivity shows the measurement of the ability of water to pass an electrical current. Conductivity of water may affected by temperature. The high temperature of water increases the conductivity of the water. The unit for electrical conductivity is the "mho" or "Siemens". Electrical conductivity is measured using probe and meter. Electrodes will be immersed into the water sample. The meter will convert the measurement and display the results

2.5.7 Oxidation Reduction Potential

Oxidation Reduction Potential (ORP) measures an aqueous system's capacity to either release or accept electrons from chemical reactions. When a system tends to accept electrons, it is an oxidizing system. When it tends to release electrons, it is a reducing system. A system's reduction potential may change upon introduction of a new species or when the concentration of an existing species changes. ORP values are used to determine water quality. The values indicate a system's relative state for receiving or donating hydrogen ions, ORP measurements are used to study that the life span of bacteria in water is strongly dependent on the ORP value (Axel, 2009).

2.6 Malaysian beach water quality studies

2.6.1 Recreational Beach Water Quality Studies

The untreated recreational bathing areas are often plagued with pollution from storm water runoff, wildlife, domestic animals, boating wastes, combined sewer overflows, and malfunctioning septic systems. Pathogens in contaminated waters can cause a wide range of diseases, including ear, nose, and throat problems, gastroenteritis, dysentery, hepatitis, and respiratory illness (Dorfman and Stoner 2007). Pollution related closings and health advisories at beaches across the country were more numerous than ever in 2006, according to the Natural Resources Defense Council's annual report on bathing beach water quality. Since bathing beach water quality varies greatly in both space and time, the effective compliance monitoring program must have adequate spatial and temporal coverage to adequately protect public health (Environmental Monitoring Assessment, 2010).

Table 2.1 shows the summary of the previous studies done on beach water. Based on the table shown below, it can be conclude that most of the studies done on the faecal indicator bacteria (FIB). These indicators are widely accepted and used in water quality studies which able to assess the level of faecal contamination in water bodies (Garrido-Perez et al. 2008). The presence of these organisms has also been used to estimate the potential human health risks of other pathogenic organisms of faecal origin.

Table 2.1: Previous studies of beach water in Malaysia

	AUTHOR (YEAR)	LOCATIONS	FINDINGS
1.	Praveena *et al.*, (2013)	Teluk Kemang beach, Port Dickson	Total coliforms concentrations were found between 20 and 1940 cfu/100 ml. E. coli concentrations were between 0 and 90 cfu/100 ml. Significant correlations were found between total coliforms and E. coli with pH, temperature and oxidation reduction potential.
2.	Hamzah *et al.*, (2011)	Port Dickson, Negeri Sembilan	The number of total coliform (TC), faecal coliform (FC), and faecal streptococci (FS) exceeded the recommended limit for recreational seawater based on US EPA 1986 standards.
3.	Abdullah *et al.*, (2011)	Five coastal area along Kelantan	The coliform bacteria detected as indicator for determination the degree of water pollution. *E. coli* is the major indicator of faecal pollution.
4.	Nuchsin *et al.*, (2003)	Port Dickson, Negeri Sembilan	The concentration of heavy metal in surface water of Port Dickson was detected. The concentration is below than Indonesian Seawater Quality Standard.

2.6.2 Importance Of Microbial Studies

Microbes which include viruses, bacteria, fungi, protozoa, and microalgae comprise most of the earth's biomass, maintain its environments, and hold the key to understanding the history of life on earth. Microorganisms used in research have many useful properties. They grow on simple, cheap and often give rise to large populations in a matter of 24 hours. It is easy to isolate their genomic material, manipulate it in the test tube and then place it back into the microbe. Due to their large populations it is possible to identify rare events and then, with the use of powerful selective techniques, isolate interesting bacterial cells and study them. These advantages have made it possible to test hypotheses rapidly

2.6.3 Water Quality Standards in Malaysia

There are several local guidelines referred related to the water quality such as Environmental Quality Acts, National Water Quality Standards for Malaysia (NWQS) and the Malaysian Water Association's (MWA). The Department of Environment (DOE) used Water Quality Index (WQI) to evaluate the status of the river water quality. The WQI serves as the basis for environment assessment of a watercourse in relation to pollution load categorization and designation of classes of beneficial uses as provided for under the NWQS.

For marine water, the Interim Marine Water Quality standards (IMWQS) have been used as the benchmark for the marine monitoring program in 1978 for Peninsular Malaysia and in 1985 for Sabah and Sarawak. The main contaminants of the coastal waters measured are total suspended solids, *Escherichia coli* and oil and grease. The standard limits for enterococci are left behind. According to U.S EPA 1986, the density of the enterococci indicators in marine water may not exceed 35 per 100mL. Therefore, a suggested approach for the currently existing Water Quality Index will be a revision that incorporates additional FIB parameters which includes the combinations of total coliform (TC), faecal coliform (FC), and faecal streatococci (FS) as useful parameters for pollution indication. The beaches can be classified as good, fair or poor based on counts obtained per 100 mL for each of these FIB (Dada *et al.,* 2012).

CHAPTER 3

METHODOLOGY

3.1 Study design

This is cross sectional study. This study was carried out at present time of study to evaluate the concentrations of *Staphylococcus aureus* and *Salmonella* in Morib beach and general perceived human health symptoms from beach water exposure.

3.2 Study location

The research was conducted at Morib beach, Banting, Selangor which located under Kuala Langat district.

Figure 3.1: Location of Morib beach

3.3 Study population

The study population was the visitors to Morib beach.

3.4 Sampling

3.4.1 Study sample

Study sample were water from Morib beach. Water samples were analyzed in the laboratory to determine the concentrations of bacterial indicators in beach water samples (*Staphylococcus aureus,* and *Salmonella spp.).* It also involved respondents who exposed beach water at Morib beach.

3.4.2 Sample size

The sample size was calculated using formula by Daniel *et al.* (1999)

$$\mathbf{n = Z^2 \times P\,(1\text{-}P) / d^2}$$

n = required sample size

Z = Z value for a given confidence level at 95% (1.96)

P = expected prevalence

d = margin of error at 5% (0.05)

Based on the study done by Prieto *et al.* (2001), the rate symptoms from exposure to marine recreational water were 0.075. Therefore, based on the value, the number of respondents required was:

$$\mathbf{n = 1.96^2 \times 0.075\,(1\text{-}0.075) / 0.05^2}$$

$$\mathbf{=106.6}$$

10% was added to the sample size to account for non-response or recording error. Therefore, the total required sample size was 117 respondents.

3.4.3 Sampling method

Simple random sampling method was applied to collect beach water samples.

3.4.4 Sampling unit

The sampling unit for questionnaire study was chosen based on the inclusion criteria:

i. Individuals with aged above 15 years old and below 65 years old.

ii. Have a good health conditions for example no dermatitis problems and diarrhoea problems.

3.5 Data collection procedure

3.5.1 Water sampling

The beach water samples from Morib were collected on weekend to indicate the busiest period at Morib beach. The beach water samples were taken 100 metres from the wash zone of beach. There were eight sampling points including two samples taken from the possible sources of pig waste effluent runoff. The distance of the beach water samples were taken depends on the boat accessibility. The sterile Schott bottles of 1L were used to collect the beach water samples. The beach water samples were taken 5 to 30 cm below the water surface by using hand dip method. The beach water samples were kept in ice or refrigerated at temperature of 1 to 4°C. The samples were analysed within 6 hour after collection

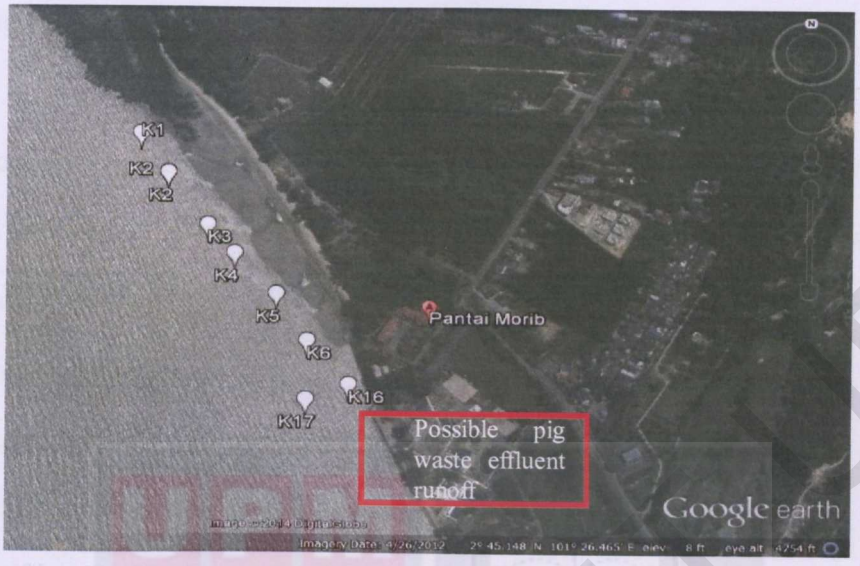

Figure 3.2: Sampling point for the water sampling and possible pig waste effluent runoff.

3.5.2 Physicochemical Parameters Measurement

All physicochemical parameters were measured on site by using YSI Profesional Plus Handheld multi parameter probe. YSI Professional Plus Handheld multi parameter probe able to monitor pH, salinity, oxidation reduction potential (ORP), temperature, and electrical conductivity and dissolved oxygen (DO). The measurements of each parameter were recorded triplicate at each point of locations. Secchi disk was used to measure turbidity of the beach water.

3.5.3 Questionnaire

A modified questionnaire adapted from previous study done by Chen *et al,* (2013) was used to get the information from the respondent. Two additional questions and symptoms were added in the questionnaires. This questionnaire was made up of four parts was showed in Appendix 1.

Section A: Socio-demographic information of study respondent

Section B: Respondent's health status

Section C: Respondent's activities and exposure behaviours on the beach

Section D: Perceived health symptoms

A pre test questionnaire was administered to 10% of the sample size. The beach visitors of Morib beach was approached to take part in the survey. The sample unit that fulfil the selection criteria was interviewed individually to answer the questions.

3.6 Laboratory analysis of isolation and enumeration of *Salmonella spp.*

3.6.1 Sample filtration

A total 100 ml of beach water samples were filtered through filtration apparatus. Sterile, gridded Whatman cellulose nitrate membrane filter with 47mm diameter and 0.45 µm pore was used to filter the water and culture the bacteria onto the selective media.

Figure 3.3: Membrane filtration manifold

3.6.2 Pre-enrichment, enrichment and subculture to selective agar

The buffered peptone water and membrane filters were gently mixed and incubated at 37 °C for 21± 3 hours. After incubation, 0.1 mL of the buffered peptone water was mixed with 10 mL of Rappaport Vassiliadis enrichment broth. The mixture were incubated at 41.5 °C for 21± 3 hours. Then, a loop of Rappaport Vassiliadis enrichment broth was plate out onto xylose lysine desoxycholate agar (XLD agar) and incubated at 37 °C for 21± 3 hours. After plating, the Rappaport Vassiliadis enrichment broth was incubated at 41.5 °C for 21± 3 hours. Steps iii and iv were repeated. After respective incubation period, the Petri dishes were examined under the light using a hand lens. The colonial appearances with smooth red colonies with black centres or wholly black colonies were examined, differentiate and calculated.

$$\text{Presumptive count/100ml} = (\text{number of colonies})/\text{volume tested} \times 100$$

3.7 Laboratory analysis of *Staphylococcus aureus*

The method used for the enumeration of *Staphylococcus aureus* was 'Enumeration of *Staphylococcus aureus* by membrane filtration'. A total 100 ml of beach water samples were filtered by using Whatman 0.45µm membrane filter. After filtration, the membrane filter was placed onto *Staphylococcus* 110 medium (Difco Laboratories, Detroid, Mich.). The medium was incubated at 35°C for 48 hours. After incubation, all colonies that were off-white through cream to yellow were enumerated as presumptive *Staphylococcus aureus*. Presumptive counts of the *Staphylococcus aureus* were calculated using formula:

$$\text{Presumptive count/100ml} = (\text{number of colonies})/\text{volume tested} \times 100$$

3.8 Ethical issue

This study was conducted on voluntary basis where the entire respondents were given briefing on conduct the experiment. Later, the entire respondents were asked to give their written permission using agreement form. All the information obtained from the study remained private and confidential for the research purpose only. This study has been approved by the Ethics Committee of Universiti Putra Malaysia before being carried out.

3.9 Quality control

i. All the physicochemical parameters such as pH, salinity, oxidation reduction potential (ORP), temperature, and electrical conductivity and dissolved oxygen (DO) were measured in triplicates by using YSI Professional Plus Handheld multi parameter probe

ii. Water samples were analyzed in triplicates to ensure the sampling and lab analysis.

iii. To maintain the quality of samples, it was kept in the ice box with temperature between 1 to 4°C.

iv. All the equipments were autoclaved before being used. This is important to prevent occurrence of any contamination.

Figure 3.4: Autoclave machines

3.9.1 Standard Operating Procedures (SOPs) and calibration

i. All the steps were carried out according to Standard Operating Procedure in the laboratory.

ii. The YSI Professional Plus hand-held multi probe was calibrated before being tested.

3.9.2 Pre-test Questionnaire

A pre-test questionnaire was conducted by the researcher and administered to different group of individual. 10% of the sample size was administered to answer the questions. This is to ensure that the questions are easily understandable. The results of pre-test were used to improve the phrasing of questions in the questionnaire.

3.10 Data analysis

The statistical analysis was done using "IBM Statistical Package for Social Sciences (SPSS) Version 22. The data was transformed and computed arithmetic to get the normal distribution data.

Table 3.1: Statistical analysis

OBJECTIVES	STATISTICAL ANALYSIS
To determine socio demographic of beach visitors in Morib beach.	Descriptive analysis
To determine and compare concentrations of *Staphylococcus aureus* and *Salmonella* at Morib beach of bathing zones with other studies.	Descriptive analysis and Comparative analysis
To determine exposure behaviour of beach visitors in Morib beach.	Descriptive analysis
To compare concentrations of *Staphylococcus aureus* and *Salmonella* between sampling points.	Comparative analysis (Kruskall-Wallis)
To determine correlations between concentrations of *Staphylococcus aureus, Salmonella* and physicochemical parameters.	Correlation test (Pearson test)
To determine commonly reported perceived health symptoms among beach visitors from bathing water exposures	Descriptive analysis

Table 3.2: Normality data of each variables

Variables	p	Normality
Temperature	0.030	Not Normal
pH	0.370	Normal
DO	0.001	Not Normal
Salinity	0.000	Not Normal
ORP	0.065	Normal
EC	0.000	Not Normal
Turbidity	0.584	Normal
Salmonella spp.	0.000	Not Normal
Staphylococcus aureus	0.005	Not Normal

Note: DO; dissolved oxygen, EC; electrical conductivity, ORP; oxidation reduction potential

CHAPTER 4

RESULTS

4.1 Response rate and socio- demographic data

The study was conducted among visitors at Morib beach. Total 117 of respondents were selected at randomly to answer the questionnaire. The questionnaire was randomly distributed to all respondents which have been selected according to inclusion criteria. The response rate was 100% in this study.

Table 4.1 and 4.2 showed the socio demography of respondents among the beach visitors at Morib beach. The variables studied were age, gender, races, place of residence, and education level. The highest group of respondents was between ages of 25 and 34 years old at 40.2% (n= 47). The least group of respondents was between ages of 45 and 64 years old at 3.4% (n= 4) only. The second highest group was from ages of 15 and 24 years old at 37.6% (n= 44) following group of ages between 35 and 44 years old 18.8% (n= 22). In this study, most of the respondents were female with 60.7% (n= 71) following male at 39.3% (n= 46). Malays were the largest proportion of ethnicity, with 71.8% (n= 84), followed with Indians 24.8% (n= 29), Chinese and others at 1.7% (n= 2). For the place of residence, the least of the visitors were from other country 1.7% (n= 2), followed by other states 21.4% (n= 25). The highest visitors to Morib beach were from Selangor at 53.0% (n= 62) and followed by locals at 23.9% (n= 28). The distribution of education level among the respondents shows that 39.3% (n= 46) of the respondents had completed SPM (secondary level), 31.6% (n= 37) had obtained their Diploma/STPM, and 18.8% (n= 22) had completed their degree level. The least education level among the respondents was at 10.3% (n= 12) for SRP/PMR level.

Table 4.1: Respondent's socio-demographic data

Socio demographic characteristics	N= 117	Percentage (%)
Age		
15-24	44	37.6
25-34	47	40.2
35-44	22	18.8
45-64	4	3.4
Gender		
Male	46	39.3
Female	71	60.7
Races		
Malay	84	71.8
Chinese	2	1.7
Indian	29	24.8
Others	2	1.7
Place of residence		
Locals	28	23.9
Selangor	62	53.0
Other states	25	21.4
Other country	2	1.7
Education level		
SRP/PMR	12	10.3
SPM	46	39.3
Diploma/STPM	37	31.6
Degree	22	18.8

4.2 Exposure behaviour

Table 4.2 shows the exposure behaviour of respondents in Morib beach. The variables studied include frequency of visit, water activities, way of exposure, duration of exposure, accidental ingestion of water and news about pollution at Morib beach.

From the result obtained, it was determined the highest frequency of visit was once every year 41.9% (n= 49), followed by once every 6 month at 26.5% (n= 31) and 23.9% (n= 28) once every month and the least frequency of visit was once every week at only 7.7% (n= 9). From the Chi-square test of contingency in Table 4.3, it shows that there was no significant relationship between the residence of visitors and the frequency of visits ($\chi^2 = 6.97$, $p > 0.05$).

For the duration of the exposure, the highest result determined the respondents were exposed for 30 minute (30.8%), while the longest duration of 2 hours 30 minutes was the least duration of exposure which only 7.7% (n= 9). A total of 53% (n= 62) of the respondents were involved in sea bathing or swimming while the lowest percentage was 5.1% (n= 6) that involved with other water activities. For the water activities, the highest activities involved was sea bathing or swimming at 53.0%. From the result obtained, it was determined 38.5% (n= 45) of the respondents were exposed whole body including head while the least exposed to waist level only 9.4% (n= 11). It was determined a group of respondents (n= 34) reported have accidently ingested the

seawater during get involved in water activities such as swimming, walking at the sea edge, water activities and others. The highest number when involved in swimming activities that is n= 20. The other 83 respondents reported never ingested the seawater during doing water activities. Only three respondents who exposed to waist level has accidently ingested the seawater. More than half of the respondents, 59.8% (n= 70) have known about the pollution at Morib beach.

Based on Table 4.4 Chi-square test of contingency between accidental ingestion of seawater and activity, it showed there was no significant relationship between the accidental ingestion of seawater with activities (χ^2= 2.70, p> 0.05). The other Chi-square test in Table 4.4 showed about the contingency between accidental ingestion of seawater and way of exposure. It was determined there was no significant relationship between way of exposure with the accidental ingestion of seawater (χ^2= 1.53, p> 0.05). Total of the respondents exposed to whole body including head 38.5% (n= 45), and 16 out of 45 was accidently ingested the seawater while the rest were not ingested the seawater.

Table 4.2 (a) : Exposure behaviour of respondents

Exposure behaviour	N= 117	Percentage (%)
Frequency of visit		
Once every week	9	7.7
Once every month	28	23.9
Once every 6 month	31	26.5
Once every year	49	41.9
Water activities		
Sea bathing/ Swimming	62	53.0
Water activities	16	13.7
Walking at the sea water edge	33	28.2
Others	6	5.1
Way of exposure		
Exposure to leg level only	33	28.2
Exposure to waist level only	11	9.4
Whole body exposure not including head	28	23.9
Whole body exposure including head	45	38.5

Table 4.2 (b): Exposure behaviour of respondents

Exposure behaviour	N= 117	Percentage (%)
Duration of exposure		
30 minutes	36	30.8
1 hour	34	29.1
1 hour 30 minutes	17	14.5
2 hours	21	17.9
2 hour 30 minutes	9	7.7
Accidental ingestion of water		
Yes	34	29.1
No	83	70.9
Information about Morib beach		
Yes	70	59.8
No	47	40.2

Table 4.3: Chi-square test of contingency between place of residence and frequency of visits

Frequency of visits	Place of residence						
	Locals	Selangor	Other states	Other country	Total	χ^2	p
Once every week	2	5	2	0	9	6.97	0.64
Once every month	7	15	6	0	28		
Once every 6 month	9	15	5	2	31		
Once every year	10	27	12	0	49		
Total	28	62	25	2	117		

Table 4.4: Chi-square test of contingency between accidental ingestion of seawater with activity and way of exposure

Variables	Accidental ingestion of seawater				
	Yes	No	Total	χ^2	p
Water activities				2.70	0. 44
Sea bathing/ Swimming	20	42	62		
Water activities	4	12	16		
Walking at the sea water edge	7	26	33		
Others	3	3	6		
Total	34	83	117		

*Significant at $p < 0.05$

Variables	Accidental ingestion of seawater				
	Yes	No	Total	χ^2	p
Way of exposure				1.53	0.68
Exposure to leg level only	8	25	33		
Exposure to waist level only	3	8	11		
Whole body exposure not including head	7	21	28		
Whole body exposure including head	16	29	45		
Total	34	83	117		

*Significant at $p < 0.05$

Table 4.5 showed the Chi-square test of contingency between information about pollution at Morib beach and duration of exposure among beach visitors. It has determined there were no significant relationship between news on pollution at Morib beach and duration of exposure (χ^2= 1.69, p> 0.79). From the Table 4.6, it was determined there were no significant relationship between information about pollution at Morib beach and frequency of visit (χ^2= 3.72, p> 0.79).

Table 4.5: Chi-square test of contingency between information about Morib beach and duration of exposure

Duration of exposure	Information about Morib beach				
	Yes	No	Total	χ^2	p
30 minutes	21	15	36	1.69	0.79
1 hours	19	15	34		
1 hour 30 minutes	11	6	17		
2 hours	12	9	21		
2 hour 30 minutes	7	2	9		
Total	70	47	117		

Table 4.6: Chi-square test of contingency between information about Morib beach and frequency of visit

Frequency of visits	Information about Morib beach				
	Yes	No	Total	χ^2	p
Once every week	5	4	9	3.72	0.29
Once every month	21	7	28		
Once every 6 month	18	17	31		
Once every year	26	23	49		
Total	70	47	117		

4.3 Concentration of *Salmonella* and *Staphylococcus aureus*

All concentrations of *Salmonella* and *Staphylococcus aureus* were analyzed in laboratory. The concentration of bacteria at each sample was determined by using descriptive statistical test.

The highest concentration of *Salmonella* was found at sampling point K17 (100.67± 82.40) while the lowest at sampling point K6 (7.00± 9.64). The second highest

concentration of the *Salmonella* was at the point K16 with mean 87.00± 40.95, and followed with 12.00± 20.78 at sampling point K4.

The highest concentrations of *Staphylococcus aureus* was detected at sampling point K16 (133.67 ±111.329), followed with 129.67±85.23 at sampling point K17 and the lowest was at sampling point K2 (13 ±13.53). Figure 4.1 showed the differences of concentrations of both *Salmonella* and *Staphylococcus aureus* at each sampling points.

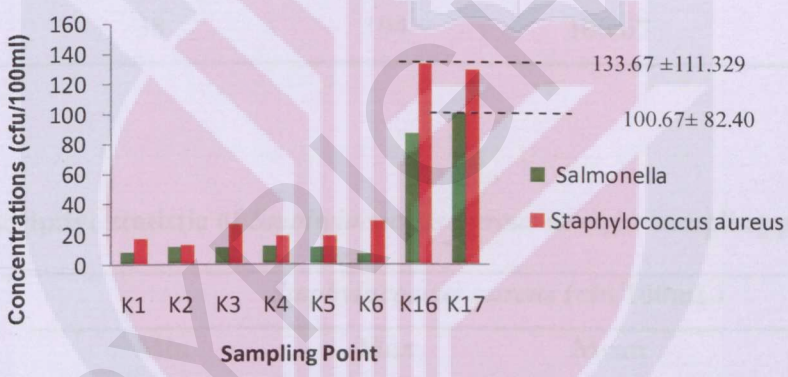

Figure 4.1: The concentrations of *Salmonella* and *Staphylococcus aureus* at eight sampling points

Table 4.7: Descriptive statistics of *Salmonella* and *Staphylococcus aureus* at eight sampling points

Sampling points	*Salmonella* (cfu/100mL)			
	Min	Max	Mean	SD
K 1	00	24	8.00	13.86
K 2	00	35	11.67	20.21
K 3	00	21	11.00	10.54
K 4	00	36	12.00	20.78
K 5	00	21	11.33	10.60
K 6	00	18	7.00	9.64
K 16	50	131	87.00	40.95
K 17	38	194	100.67	82.40

Table 4.8: Descriptive statistic of *Staphylococcus aureus* at eight sampling point

Sampling points	*Staphylococcus aureus* (cfu/100mL)			
	Min	Max	Mean	SD
K 1	00	39	17	19.97
K 2	00	27	13	13.53
K 3	00	46	26.67	23.86
K 4	5.00	40	19	18.52
K 5	00	56	18.67	32.33
K 6	00	56	28.67	28.02
K 16	43	242	133.67	111.33
K 17	32	189	129.67	85.23

4.4 Physicochemical parameters measurements

Physicochemical parameters such as temperature, pH, salinity, turbidity, electrical conductivity (EC), dissolved oxygen (DO), and oxidation reduction potential (ORP) were measured and recorded on site. Table 4.9 showed the physicochemical parameters measurements at eight sampling points

From the recorded data, it was determined there was an increasing trend for temperature at each sampling point. The lowest temperature recorded were 29.27 ± 0.67 at sampling 6 and the highest temperature determined was 30.93 ± 0.06 at sampling point K17, followed by 30.53 ± 0.12 at sampling point K16. pH of the seawater ranged from 7.76 ± 0.06 to 8.28 ± 0.01. At sampling point K16, the pH of the seawater was slightly neutral, 7.76 ± 0.06, while at sampling point K5, K6 were same at 8.28 ± 0.01. The lowest reading of dissolve oxygen (DO) was at sampling point K1 (4.58 ± 0.018), then followed by 4.59 ± 0.01 at sampling point K6. The highest DO value recorded was 6.17 ± 0.11at sampling point K17.

Next, the electrical conductivity (EC) in water were measured and found the highest value was at sampling point K6 (50047.3 ± 10.12), followed by 49988.7±2.08 at K4. The lowest level was recorded at sampling point K 17 (43716.33±6055.4), while the highest salinity level was recorded at point K6 (32.63 ± 0.04). For the oxidation

reduction potential (ORP), the reading at sampling point K17 showed the lowest value 757.53 ±0.15.

The reading was recorded high at sampling point K5 and K6 (757.90 ± 0.06 and 757.80 ±0.00). Lastly, turbidity level at sampling point K5 was the lowest, which was 2.05 ± 0.02, followed by sampling point K16 (2.09 ± 0.01), and followed by sampling point K4 (2.10 ± 0.02). Turbidity reading at point K1 was the highest 2.29 ± 0.02.

4.4 Physicochemical parameters measurements

Table 4.9: Mean and standard deviation of physicochemical parameters measurements at eight sampling points

Sampling point	Temperature (°C)	pH	DO (mg/l)	EC (mS/cm)	Salinity (ppt)	ORP (mV)	Turbidity (NTU)
			Mean ± SD				
K 1	29.60 ± 0.27	7.95 ±0.31	4.58 ±0.18	49909.0±13.11	32.45 ±0.13	757.70 ±0.00	2.29 ± 0.02
K 2	29.77 ± 0.06	8.11 ±0.08	4.63 ± 0.04	49914.3±16.74	32.55 ± 0.01	757.70 ± 0.06	2.21 ± 0.02
K 3	29.67 ± 0.06	8.17 ±0.04	4.60 ± 0.01	49947.3±1.16	32.56 ± 0.00	757.70 ± 0.0	2.15 ± 0.02
K 4	29.77 ± 0.06	8.18 ±0.01	4.69 ± 0.01	49988.7±2.08	32.60 ± 0.00	757.80 ± 0.0	2.10 ± 0.02
K 5	29.83 ± 0.12	8.28 ±0.01	4.72 ± 0.02	49925.0±11.00	32.54 ±0.01	757.90 ± 0.00	2.05 ± 0.02
K 6	29.27 ± 0.67	8.28 ±0.01	4.59 ± 0.01	50047.3±10.12	32.63 ± 0.04	757.90 ± 0.06	2.12 ± 0.02
K 16	30.53 ± 0.12	7.76± 0.06	5.36 ± 0.02	49883±12.12	32.46 ± 0.00	757.80 ±0.00	2.09±0.01
K 17	30.93 ± 0.06	8.10 ±0.03	6.17 ± 0.11	43716.33±6055.4	31.29 ± 1.13	757.53 ±0.15	2.15±0.01

4.5 Normality test of variable

Table 4.10 shows the result of normality for all variables. Shapiro-wilk normality test shows that the data for temperature, pH, salinity, DO, EC, ORP and concentration of *Staphylococcus aureus* variables were not normally distributed ($p<0.05$). Therefore, the data was transformed and computed arithmetic to get the normal distribution data. Only two variables turbidity and pH have normal distribution ($p>0.05$).

Table 4.10: Shapiro- wilk test for normality for variables

Variables	p	Normality
Temperature	0.030	Not Normal
pH	0.370	Normal
DO	0.001	Not Normal
Salinity	0.000	Not Normal
ORP	0.065	Normal
EC	0.000	Not Normal
Turbidity	0.584	Normal
Salmonella	0.000	Not Normal
Staphylococcus aureus	0.005	Not Normal

Note: **DO; dissolved oxygen, EC; electrical conductivity, ORP; oxidation reduction potential**

4.6 Comparison of *Salmonella* and *Staphylococcus aureus* between sampling points

Based on the normality test done (Shapiro-Wilk), it showed both concentrations of *Salmonella* and *Staphylococcus aureus* were not normally distributed, ($p<0.05$). Therefore, Kruskall-Wallis non-parametric wer used to compare both concentrations of *Salmonella* and *Staphylococcus aureus* at eight sampling points.

According to test analysis, it showed there were no significant differences in concentrations of *Salmonella* ($H= 11.78$, $p= 0.11$), and no significant differences in concentrations of *Staphylococcus aureus* ($H= 9.63$, $p=0.21$) between sampling points.

Table 4.11: Kruskal-wallis test to compare concentration of *Salmonella* and *Staphylococcus aureus* between eight sampling points

Sampling point	N	Mean rank	H	p
	Salmonella		11.78	0.11
K 1	3	8.67		
K 2	3	10.00		
K 3	3	10.17		
K 4	3	10.33		
K 5	3	10.50		
K 6	3	9.33		
K 16	3	18.00		
K 17	3	23.00		
	Staphylococcus aureus		9.63	0.21
K 1	3	9.17		
K 2	3	7.83		
K 3	3	11.83		
K 4	3	10.67		
K 5	3	9.00		
K 6	3	11.83		
K 16	3	20.33		
K 17	3	19.33		

4.7 Correlation between concentration of *Salmonella spp.*, *Staphylococcus aureus* and physicochemical parameters

Result of normality test performed by using Shapiro-wilk showed data for temperature, salinity, DO, EC, concentrations of *Salmonella and Staphylococcus aureus* variables were not normally distributed ($p < 0.05$). There were only three variables were normal distributed ($p > 0.05$), which were pH, ORP and turbidity. All not normally distributed variables were transformed and computed arithmetic logarithm to get the normal distribution data. Thus, the Pearson correlation coefficient test was performed to obtain the relationship between the concentrations of *Salmonella, Staphylococcus aureus* and physicochemical parameters.

Growth and activities of *Salmonella* and *Staphylococcus aureus* was affected by physicochemical parameters. Thus, physicochemical parameters were measured to identify the correlation between concentration of *Salmonella* and *Staphylococcus aureus*. Based on the result obtained, it showed there were significant relationships between concentrations of *Salmonella, Staphylococcus aureus* and physicochemical parameters (salinity, temperature, dissolved oxygen and electrical conductivity). There were significant relationship between concentrations of *Salmonella* and temperature ($r = 0.96$, $p < 0.01$).There were also significant relationship between concentrations of *Salmonella* with dissolved oxygen (DO) and salinity ($p<0.05$). Strong positive correlation was found between concentrations of *Salmonella* and DO ($r = 0.94$, $p<0.01$). There was significant

relationship between concentration of *Salmonella* with salinity ($r = -0.74$, $p < 0.05$). It showed negative correlation between both variables.

The results determined there were significant correlations between concentrations of *Staphylococcus aureus* with temperature, dissolved oxygen (DO), salinity and electric conductivity (EC). There was strong positive correlation between concentrations of *Staphylococcus aureus* with temperature ($r = 0.99$, $p < 0.01$), and DO ($r = 0.96$, $p < 0.01$). Apart from that, there was significant relationship between concentrations of *Staphylococcus aureus* with salinity ($r = -0.80$, $p < 0.05$). The value of Pearson coefficient showed the weak negative correlation. For the electrical conductivity (EC), there was significant relationship between both variables which are concentrations of *Staphylococcus aureus* and EC ($r = -0.78$, $p < 0.05$).

Table 4.12: Correlation between the concentration of *Salmonella spp.*, *Staphylococcus aureus* and physicochemical

Variables	Turbidity	pH	DO	Salinity	ORP	EC	Temperature	*Salmonella*	*Staphylococcus aureus*
Turbidity	1	-0.31	-0.17	-0.08	-0.75*	-0.03	-0.31	-0.19	-0.26
pH		1	-0.34	0.14	0.43	0.05	-0.34	-0.59	-0.41
DO			1	-0.91**	-0.47	-0.90**	0.97**	0.94**	0.96**
Salinity				1	0.67	0.99**	-0.81*	-0.74*	-0.80*
ORP					1	0.61	-0.29	-0.36	-0.32
EC						1	-0.81*	-0.70	-0.78*
Temperature							1	0.96**	0.99**
Salmonella								1	0.97**
Staphylococcus aureus									1

****Significant at p<0.01* Significant at p<0.05**

Note: **DO; dissolved oxygen, EC; electrical conductivity, ORP; oxidation reduction potential**

4.8 Perceived health symptoms

In the questionnaire, the beach visitors were asked about any perceived health symptoms caused by the exposure to the bathing water at Morib beach. Table 4.13 shows the perceived health symptoms among the respondents at Morib beach.

Based on the result obtained, it was determined that 56.4% (n= 66) of the respondents has none experienced perceived health symptoms such as gastroenteritis, Acute febrile respiratory illness (AFRI), skin symptoms, eye symptoms, ear symptoms, and enteric fever.

Total 31.6% (n= 37) of the respondents have experienced skin symptoms such as skin irritation, dryness or itchiness. Other 4.3% (n= 5) respondents were experienced from ear symptoms while both acute febrile respiratory illness (AFRI) and eye symptoms were only 2.6% (n= 3). Enteric fever showed low numbers of perceived health symptoms that only 1.7% (n= 2). The least perceived health symptoms experienced determined was as gastroenteritis which only 0.9% (n= 1).

Table 4.13: Perceived health symptoms among respondents

Health symptoms	Yes		No	
	N	Percentage (%)	N	Percentage (%)
Gastroenteritis	1	0.9	117	99.1
Acute febrile respiratory illness (AFRI)	3	2.6	114	97.4
Skin symptoms	37	31.6	80	68.4
Eye symptoms	3	2.6	114	97.4
Ear symptoms	5	4.3	112	95.7
Enteric fever	2	1.7	115	98.3
None	66	56.4	51	43.6

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Socio-demographic data

The questionnaire was randomly distributed to 117 respondents which have been selected according to inclusion criteria. It was determined the highest group of

respondents were between ages of 25 and 34 years old. At this group of age is more active and physically fit compared to other group of ages. The least group of respondents were between ages of 45 and 64 years old. The high group of ages was less active as increasing their ages.

The highest numbers of respondents were female compared to male. For the ethnicity, Malays were the largest proportion followed with Indians, Chinese and others. The highest visitors to Morib beach were from Selangor because Morib beach was located only 10 km 15 km from the town of Banting. It was determined the lowest number of visitors was from other country. Visitor from other country may not know well about Morib beach because it was established and developed past three years. The distribution of education level among the respondents shows most of the respondents had completed secondary level (SPM).

5.1.2 Exposure behaviour

For exposure behaviour of respondents in Morib beach the variables studied include frequency of visit, water activities, way of exposure, duration of exposure, accidental ingestion of water and news about Morib beach.

The visitors from Selangor showed the highest percentage frequency of visit which was once every year. The strategic location of Morib beach near to town of Banting and equipped with excellent facilities have promotes it as the famous recreational park. Morib beach was found as one of the nicest beaches can be found along Selangor's coastline.

The risk of the health symptoms were affected by the duration of the exposure (Mugglestone *et al.,* 2000). From the result obtained, it showed most of the respondents were exposed for 30 minutes to beach water. The longest duration of exposure was 2 hour 30 minutes. The long duration of exposure may increase the risk to health symptoms. The highest activity involved was sea bathing or swimming. Most of the respondents were exposed whole body including head. Some studies have shown that swimmers were at a higher risk of getting illness compared to non-swimmers. The swimmers were at a greater risk when swimming at beaches affected by pollution compared with beaches that were considered to be unpolluted (Petrilli *et al.* 1980; Fleisher *et al.* 1996). According to Colford et al., 2007, a study done in Mission Bay

found that swimmers that having water contact experienced more diarrhea and skin rash compared to non- swimmers.

About 29.1% (34) of the respondents reported have accidently ingested the water. Ingestion of polluted water may cause the incidence of gastroenteritis. Water was accidently ingested during involved in swimming activities. The incidence of skin rash and diarrhoea were mediated by water contact. These symptoms may increase with great exposure categories such as swallowing water (Colford et al., 2007).

5.1.3 Concentration of *Salmonella spp.* and *Staphylococcus aureus*

The water samples were collected at eight locations in Morib beach. The concentrations of *Salmonella* and *Staphylococcus aureus* were analyzed in laboratory.

The presence of *Salmonella* in water may cause potential threat to human health. These bacteria can cause foodborne and waterborne outbreaks (Ruiz *et al.* 1995; Usera *et al.* 1995; Clark et al. 1996; Kramer *et al.* 1996). The study detected range concentrations of *Salmonella* were 0 cfu/100mL to 194 cfu/100mL. Acocrding to European Community Bathing Water Directive (EEC 1976), a *Salmonella* standard of nil in 1 liter of water have established, but does not recommend the means by which *Salmonella* should be concentrated and detected. The directive's standard for *Salmonella* is mandatory,

requiring a 95% compliance level of waters during the recognized bathing season. This approach seems insufficient to determine the health-hazard of *Salmonella* in waters (Figueras *et al.* 1997; Polo et al. 1998). Therefore, the other countries who conduct the monitoring of the bathing waters have used other microbiological parameters.

The concentrations of *Staphylococcus aureus* was found to ranged between 0 cfu/100ml to 189 cfu/100ml. A study by Plano (2013) found that the isolation of *Staphylococcus aureus* was at range of 2–780 CFU/100 mL water. There were increasing of concentrations of *Staphylococcus aureus* associated with marine environments and recreational beaches (Soge *et al.*, 2009; and Levei-E.E *at al.,* 2012). However, none have been associated with reported outbreaks of infections.

In Malaysia, there are several local guidelines referred related to the water quality such as Environmental Quality Acts, National Water Quality Standards for Malaysia (NWQS) and the Malaysian Water Association's (MWA). For marine water, the Interim Marine Water Quality standards (IMWQS) have been used as the benchmark for the marine monitoring program in 1978 for Peninsular Malaysia and in 1985 for Sabah and Sarawak. However, the main contaminants of the coastal waters measured are total suspended solids, *Escherichia coli* and oil and grease. There was no standard for concentrations of *Staphylococcus aureus* and *Salmonella.*

Based on the existing standard, U.S EPA (1986), the density of the enterococci indicators in marine water may not exceed 35 per 100mL. Therefore, a suggested approach for the currently existing Water Quality Index will be a revision that incorporates additional FIB parameters which includes the combinations of total coliform (TC), faecal coliform (FC), and faecal streatococci (FS) as useful parameters for pollution indication. The beaches can be classified as good, fair or poor based on counts obtained per 100 mL for each of these FIB (Dada *et al.*, 2012).

5.1.4 Comparison of *Salmonella spp.* and *Staphylococcus aureus* between sampling points

The water samples were taken at eight locations in Morib beach. At sampling point of K1 to K6, the water samples were taken 100 meters from the sea edge, while other two samples (K16 and K17) were taken at possible pig farming waste effluents runoff. From the result obtained, it was found the highest concentrations of both *Salmonella* and *Staphylococcus aureus* were at the possible pig farming waste effluents. According to Utusan Malaysia (2008), it reported that pig farming activities in Ladang Tumbuk, Selangor has resulted in surface and beach water pollution surrounding the area. Ladang Tumbuk is located three to five km from Morib Gold Coast has released the pig farm waste direct to river.

Total concentrations of *Staphylococcus aureus* are higher than *Salmonella.* This might be caused by the highest numbers of bathers who contributes to high number of *Staphylococcus aureus.* A study by Elmir S.M et al., 2007 found that approximately 10^5 CFU of *Staphylococcus aureus* could be released into the water by single bather. *Staphylococcus aureus* can be found mainly on the skin and mucous membranes of animals. It also occasionally detected in the gastrointestinal tract and sewage (LeChevallier MW and Seidler RJ (1980).

5.1.5 Correlation between *Salmonella, Staphylococcus aureus* and physicochemical parameters

Normality test from Shapiro-wilk shown data for temperature, salinity, DO, EC, concentrations of *Salmonella and Staphylococcus aureus* variables were not normally distributed ($p < 0.05$). There were only three variables were normal distributed ($p > 0.05$), which were pH, ORP and turbidity. All not normally distributed variables were transformed and computed arithmetic logarithm to get the normal distribution data. Thus, the Pearson correlation coefficient test was performed to obtain the relationship between the concentrations of *Salmonella, Staphylococcus aureus* and physicochemical parameters.

Growth and activities of *Salmonella* and *Staphylococcus aureus* was affected by physicochemical parameters. Based on the result obtained, it showed there is significant relationship between concentrations of *Salmonella, Staphylococcus aureus* and physicochemical parameters. Based on the result obtained, it showed there were significant relationship between concentrations of *Salmonella* and temperature ($r = 0.96$, $p < 0.01$). The correlation test indicates there was strong positive correlation between these two variables. The increasing temperature will increase the concentrations of *Salmonella.* According to Shibata *et al.,* 2004, some of the physicochemical parameters changed between seasons. The temperature during wet seasons was between 30.7°C to 25.6°C, while salinity was lower at 30.0‰ and 34.4‰ on average than during the dry

season. A negative correlation between concentrations of *Salmonella* and salinity showed the higher salinity will decrease the concentrations of *Salmonella.* Lee *et al.,* 2010 stated that *Salmonella* poses differential survival response in relation to salinity. It has been reported that showed very low survival in sea water.

There was strong positive correlation between concentrations of *Staphylococcus aureus* with temperature ($r = 0.99$, $p < 0.01$), and DO ($r = 0.96$, $p < 0.01$).It showed the strong positive correlation between concentrations of *Staphylococcus aureus* with temperature. When temperature increased, the concentrations of *Staphylococcus aureus* also increase. There was strong positive correlation between concentrations of *Staphylococcus aureus* with DO ($r = 0.96$, $p < 0.01$). When dissolved oxygen increase, it will increase the level of DO. This is because *Staphylococcus aureus* are able to grow aerobically and anaerobically. Therefore, the growth of *Staphylococcus aureus* does not need oxygen do not affect level of DO.

5.1.6 Perceived health symptoms

Exposure to polluted seawater may cause illness to human health. According to Shuval, 2003 here have been 50 million reported cases of severe respiratory disease each year and 120 millions cases of gastrointestinal illness caused by polluted water. Most of these illnesses due to the water polluted with faecal waste. There was also study conducted at the beach in Miami, Florida found a relationship between levels of indicator bacteria in the water and health effects, (Fleisher *et al.,* 2010). In this study, it was determined 31.6% (37) of the respondents were experienced skin symptoms which includes itching of the skin, small reddish pimples, and tingling. According to WHO, 2001, skin, respiratory, ear, and other ailments are potentially associated with exposure to contaminated waters. There were several studies conducted on the skin related symptoms with the exposure to recreational water. A study on surfers who exposed to polluted beaches was done by Dwight *et al.*, 2004. Surfers were interviewed at two different beaches and asked about symptoms in past three months. According to A.B. Boehm and J.A. Soller (2013), *Staphylococcus* causes skin infections.

Results showed the highest percentage of perceived health symptoms were none detected which were 56.4% (n= 66). There were no perceived symptoms experienced from the beach visitors who exposed to Morib beach water. This study suggested that perceived human health symptoms among beach bathers may associated with other factors as supported by Colford *et al.,* 2007. The study from Colford *et al.,* (2007) stated

that the exceeding of California's marine recreational water quality thresholds for traditional fecal indicator bacteria was not associated with increased risk of illness. It was also suggested that these result may caused by lack of sources of faecal indicators bacteria.

5.2 Conclusion

As conclusion, this cross sectional study has determined the highest concentrations of *Salmonella* and *Staphylococcus aureus* were found near the pig waste effluent runoff. None of perceived health symptoms from beach water exposure was observed. There were some limitations during conducting the study. There was information bias since major determinants of bathing related illness are based on questionnaires and depends on the respondents answers. Next, high tides of sea water during water sampling may vary the concentrations of bacteria.

5.3 Recommendation

Further monitoring can be conducted in Morib beach to evaluate and monitors the quality of the beach water. The testing of other bacterial can be conducted to know exact sources of pollution. Next, research on the actual health symptoms due to exposure of Morib beach can be done by follow up one week after bathers.

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APPENDIX 1: QUESTIONNAIRE

No. Ref.: _____

Borang Soal Selidik / *Questionnaire Interview Form*

A. Maklumat Latar Belakang Responden / *Background Information of Respondent*

1. **Umur** / *Age*:
   ☐ **15-24** ☐ **24-34** ☐ **35-44** ☐ **45-54** ☐ **65+**
2. **Jantina** / *Gender*:
   ☐ **Lelaki** / *Male* ☐ **Perempuan** / *Female*
3. **Bangsa** / *Ethnic*:
   ☐ **Melayu** / *Malay* ☐ **Cina** / *Chinese* ☐ **India** /*Indian* ☐ **Lain-lain** /others
4. **Asal** / Place of Residence:
   ☐ **Pengunjung dari negara lain** / *Visitor from other countries* (**Negara** / *Country*: _____)
   ☐ **Pengunjung dari negeri lain** / *Visitor from other states* (**Negeri** / *States*: _____)
   ☐ **Orang tempatan Selangor** / *Local residence of Selangor* (**Kampung** /*Village*: _____)
5. **Tahap Pendidikan** / *Education Level*:
   ☐ **Sekolah rendah** / *primary level* ☐ **SRP/PMR** / *lower secondary level*
   ☐ **SPM** / *higher secondary level* ☐ **Diploma/STPM** / *college* ☐ **Ijazah** / *degree*

B. Status Kesihatan/ *Health Status*

1. **Adakah anda sedang menghadapi penyakit kronik seperti penyakit kronik dan penyakit perut?** *Do you have any chronic heath conditions such as dermatitis and frequent diarrhea*? **Jika ya, sila nyatakan** / *If yes, please state:* _____
   ☐ **Ya** / *Yes* ☐ **Tidak** / *No*
2. **Adakah anda kerap mengambil makanan mentah atau separuh mentah (e.g makanan BBQ di pantai)?** *Do you frequently consume raw or half cooked food (e.g BBQ food on the beach)?*
   ☐ **Ya** / *Yes* ☐ **Tidak** / *No*

No. Ref.: _____

C. Maklumat Pendedahan Air Laut & Aktiviti Pantai / *Water Exposure & Beach Activities Information*

1. **Kekerapan mengunjungi Pantai Morib**/ *Frequency of visit to Morib public beach*

   ☐ **1 setiap minggu** / *1 every week* ☐ **1 setiap bulan** / *1 every month*

   ☐ **1 setiap 6 bulan** / *1 every 6 month* ☐ **1 setiap tahun** / *1 every year*

2. **Aktiviti yang kerap dijalankan di pantai**/ *Water activities that are frequently done on the beach.*

   ☐ **Mandi/Berenang**/ *Sea Bathing/ Swimming*

   ☐ **Aktiviti laut**/ *Water activities information*

   ☐ **Berjalan dipersisiran laut dengan air bersentuhan pada paras kaki sahaja** / *Walking at the sea water edge with contact with seawater at feet level only*

   ☐ **Lain-lain**/ *Others*: _____

3. **Cara pendedahan kepada air pantai**/ *Exposure to water in beach*

   ☐ **Pendedahan sehingga paras kaki sahaja**/ *Exposure to leg level only*

   ☐ **Pendedahan sehingga paras pinggang sahaja**/ *Exposure to waist level only*

   ☐ **Pendedahan seluruh badan tidak termasuk kepala**/ *Whole body exposure not including head*

   ☐ **Pendedahan seluruh badan termasuk kepala**/ *Whole body exposure including head*

4. **Biasanya berapa lamakah anda terdedah kepada air laut semasa aktiviti?**/ *Usually how long are you exposed to the water during activities?*

   ☐ **30 Minit** / *30 Minutes* ☐ **1 Jam** / *1 Hour* ☐ **1 Jam 30 Minit** / *1 Hour 30 Minutes*

   ☐ **2 Jam** / *2 Hours* ☐ **2 Jam 30 Minit** / *2 Hours 30 Minutes*

5. **Adakah anda terminum air laut semasa aktiviti (e.g percikan air)** *Do you accidently swallow seawater during activities (e.g. water splashes)*

   ☐ **Ya** / *Yes* ☐ **No** / *Tidak*

6. **Adakah anda pernah mendengar berita berkenaan pencemaran Pantai Morib daripada sisa buangan haiwan ternakan sebelum ini?**/ *Have you ever heard any news on Morib beach pollution from waste livestock before?*

   ☐ **Ya** / *Yes* ☐ **No** / *Tidak*

No. Ref.: _____

D. Pengalaman Tanda-tanda Kesihatan Daripada Pendedahan Kepada Air Laut Pantai Morib/
Experience of Health Symptoms After Exposure to The Seawater in Morib Public Beach

1. **Adakah anda pernah mengalami tanda-tanda yang dinyatakan dibawah selepas pendedahan air Pantai Morib?** */ Have you ever experienced these symptoms after exposure to the water at Morib beach?* **(boleh tanda lebih daripada 1 jawapan/** *can tick more than 1 answer*)

   ☐ **Gastroenteritis (muntah, loya, cirit birit, sakit perut)** */ Gastroenteritis (vomiting diarrhea, nausea, stomach ache)*

   ☐ **Jangkitan kulit (gatal, dermatitis)** */ Skin symptoms (itchy, rash)*

   ☐ **Jangkitan mata (gatal, tidak selesa, kemerahan)** */ Eye symptoms (eye sore, irritation or redness)*

   ☐ **Jangkitan hidung (sakit sakit, keluar bendalir hidung)** */ Nose symptoms (noseache, nose discharge)*

   ☐ **Penyakit pernafasan akut (Afri) (demam, menggigil, sejuk, batuk kering, hilang selera makan, sakit badan dan loya)** */ Acute febrile respiratory illness (AFRI) (fever,shivering, chills, dry cough, loss appetite, body aches and nausea)*

   ☐ **Demam (demam teruk dan cirit birit)**/ *Enteric fever (severe fever and diarrhoea)*

   ☐ **Tiada**/ None

2. **Apakah pandangan anda akan kualiti air di Pantai Morib?**/ *What rating would you give to the water quality in Morib public beach*

   ☐ **Sangat Baik** /*Very Good* ☐ **Baik** / *Good* ☐ **Biasa** / *Fair*

   ☐ **Tidak Memuaskan** / *Bad* ☐ **Teruk** / *Very Bad*

Terima kasih atas bantuan anda.
Thank you for your help

APPENDIX 2:
FORM B2: RESPONDENT'S INFORMATION SHEET AND PARENT/GUARDIAN CONSENT

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM B2: RESPONDENT'S INFORMATION SHEET AND PARENT/GUARDIAN CONSENT

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE:

Bacterial indicators of public beach water quality in Morib beach, Selangor.

2. INTRODUCTION:

Presence of infectious microorganisms in recreational water can cause human health problems. Faecal pollution that contains pathogenic microorganisms is derived from human sewage or animal sources. Free living pathogenic microorganism exists in recreational water may cause gastrointestinal illness, health symptoms of nose, eyes, ears, and skin. Despite protecting public health, bacteriological monitoring of beach also helps in reducing bather's exposure to biological pathogenic bacteria, help in developing control measures and to access the suitability of current corrective measure. The objective of this study is to measure the concentration of bacterial indicators (*Staphylococcus aureus* and *Salmonella*) in Morib beach bathing waters and their general perceived human health symptoms from beach water exposure. The result obtained become the scientific prove whether the beach are contaminated or not.

3. WHAT WILL YOU HAVE TO DO?

You will have to answer a questionnaire regarding to this study.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

- Individual less than 15 years old

5. WHAT WILL BE BENEFITS OF THE STUDY?

(a) TO YOU AS THE SUBJECT?

This study will give you the information on the safety of water in term of bacterial quality in Morib beach of bathing zone. The questionnaire will give information on the significance of the health effects from the exposure.

b) TO THE INVESTIGATOR?

This study will help the investigator to determine the relationship between level of *Stapthylococcus aureus* and *Salmonella* in Morib beach with the implications from the exposure to the water.

6. WHAT ARE THE POSSIBLE RISKS?

There is no risk available in the study.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

The information and identity used in this study will remain confidential.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

MUNASHAMIMI BINTI HAMDAN (+6013-4335595) - Research investigator

DR. SARVA MANGALA PRAVEENA (+6016-5169081) - Research Supervisor.

*Please initial here if you have read and understood the contents of this page*_____

9. GUARDIAN'S / PARENT'S CONSENT

I ..... Identity Card No. .....

address.....

.....hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature ..... Signature .....
(Respondent) (Witness)

Date :..... Name :.....

I/C No. :.....

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date ..... Signature .....
(Researcher)

APPENDIX 3:
BORANG B1: PENERANGAN DAN PERSETUJUAN RESPONDEN

JAWATANKUASA ETIKA UNIVERSITI UNTUK
PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR, MALAYSIA

BORANG B1: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1. TAJUK KAJIAN

Bakteria penunjuk kualiti air pantai awam di Pantai Morib, Selangor.

2. PENGENALAN

Kehadiran mikroorganisma berjangkit dalam air rekreasi boleh menyebabkan masalah kesihatan manusia. Pencemaran najis yang mengandungi mikroorganisma patogen berasal dari sisa kumbahan manusia atau haiwan. Mikroorganisma patogen yang wujud di dalam air rekreasi boleh menyebabkan penyakit gastrousus, gejala kesihatan hidung, mata, telinga, dan kulit. Pemantauan bakteriologi di pantai membantu dalam mengurangkan pendedahan perenang terhadap bakteria patogenik. Ia juga membantu melindungi kesihatan awam serta membantu dalam membangunkan langkah-langkah kawalan dan langkah pembetulan semasa. Kajian ini bertujuan untuk mengukur kepekatan petunjuk bakteria (*Staphylococcus aureus dan Salmonella*) di perairan Pantai Morib serta melihat gejala kesihatan mausia yang terdedah kepada air pantai. Keputusan yang diperolehi menjadi saintifik membuktikan sama ada pantai yang tercemar atau tidak.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Responden diminta untuk menjawab soal selidik untuk mendapatkan maklumat data mengenai kajian itu.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

- Individu dengan usia di bawah umur 15 tahun

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Kajian ini akan memberi anda maklumat mengenai keselamatan air dari segi kualiti bakteria di Pantai Morib. Soal selidik ini akan memberikan maklumat mengenai kepentingan kesan kesihatan daripada pendedahan.

b) KEPADA PENYELIDIK?

Kajian ini akan membantu penyelidik dalam menentukan hubungan antara tahap *Stapthylococcus* aureus dan *Salmonella* di Pantai Morib serta implikasi daripada pendedahan kepada air.

6. ADAKAH IA BERISIKO?

Tiada risiko disediakan dalam kajian ini.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Maklumat dan identiti yang digunakan dalam kajian ini akan kekal sulit.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

MUNASHAMIMI BINTI HAMDAN (+6013-4335595) - Penyelidik

DR. SARVA MANGALA PRAVEENA (+6016-5169081) - Penyelia penyelidikan kajian

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _____

9. PERSETUJUAN

Saya..... No Kad Pengenalan. .....
beralamat.....
.....dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imei/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan .....
(*Responden*)

Tarikh :.....

Tandatangan .....
(*Saksi*)

Nama :.....

No. K/P: .....

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh .....

Tandatangan .....
(*Penyelidik*)

APPENDIX 4:
BORANG B2:PENERANGAN DAN PERSETUJUAN IBUBAPA/PENJAGA

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PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR, MALAYSIA

BORANG B2: PENERANGAN DAN PERSETUJUAN IBUBAPA/PENJAGA

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3. APAKAH YANG PERLU ANDA LAKUKAN?

Responden diminta untuk menjawab soal selidik untuk mendapatkan maklumat data mengenai kajian itu.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

- Individu dengan usia di bawah umur 15 tahun

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANAK/JAGAAN SEBAGAI PESERTA?

Kajian ini akan memberi anda maklumat mengenai keselamatan air dari segi kualiti bakteria di Pantai Morib. Soal selidik ini akan memberikan maklumat mengenai kepentingan kesan kesihatan daripada pendedahan.

b) KEPADA PENYELIDIK?

Kajian ini akan membantu penyelidik dalam menentukan hubungan antara tahap *Stapthylococcus* aureus dan *Salmonella* di Pantai Morib serta implikasi daripada pendedahan kepada air.

6. ADAKAH IA BERISIKO?

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7. ADAKAH MAKLUMAT DAN IDENTITI ANAK/JAGAAN SAYA KEKAL RAHSIA?

Maklumat dan identiti yang digunakan dalam kajian ini akan kekal sulit.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

MUNASHAMIMI BINTI HAMDAN (+6013-4335595) - Penyelidik

DR. SARVA MANGALA PRAVEENA (+6016-5169081) - Penyelia penyelidikan kajian

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _____

9. PERSETUJUAN

Saya..... No Kad Pengenalan. .....
beralamat.....
.....dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

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*potong yang tidak berkenaan

Tandatangan .....
(*Responden*)

Tarikh :.....

Tandatangan .....
(*Saksi*)

Nama :.....

No. K/P: .....

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh .....

Tandatangan .....
(*Penyelidik*)