PREVALENCE AND CHARACTERIZATION OF *ESCHERICHIA COLI* IN KELANTAN RIVER AND ITS ADJACENT COASTAL WATERS

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

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ABSTRACT

The presence of *Escherichia coli* (*E. coli*) as fecal indicator bacteria in river and seawater may cause mild to serious infections and constitutes a public health risk. Water samples were collected from 15 sites along the Kelantan River, estuaries and its adjacent coastal waters to examine the occurrence and diversity of *E. coli*. The abundance of *E. coli* ranged from 3.1 x 10 to 1.6 x 10^5 CFU 100 mL^{-1}, and total suspended solids correlated significantly with *E. coli* abundance ($r^2 = 0.165$, $p < 0.001$) and rainfall ($r^2 = 0.342$, $p < 0.001$). Phylogenetic group B1 (29.7%) and A (29.4%) were the most prevalent whereas groups B2 and D were least abundant. The higher abundance of phylogenetic group D detected at upstream sites of Kelantan River suggested fecal contamination mainly of animal origin. Canonical correlation analysis showed that phylogenetic group B2 seemed to thrive in water with higher dissolved oxygen levels whereas phylogenetic groups A and D were greater in waters with higher inorganic nutrients e.g. NH_4, NO_2 and NO_3 whereas phylogenetic group B1 appeared to have better salinity tolerance amongst phylogenetic groups.

Keywords: *E. coli*, fecal pollution, Kelantan River, phylogenetic groups

KELAZIMAN DAN CIRI-CIRI *ESCHERICHIA COLI* DI SUNGAI KELANTAN DAN AIR PANTAI BERSEBELAHANNYA

ABSTRAK

Kehadiran *Escherichia coli* (*E. coli*) sebagai bakteria penunjuk pencemaran feses di sungai dan air laut boleh menyebabkan jangkitan ringan hingga serius dan merupakan risiko kesihatan awam. Sampel air dikumpulkan dari 15 lokasi di sepanjang Sungai Kelantan, estuari dan perairan pesisirnya yang berdekatan untuk menyiasat kejadian dan kepelbagaian *E. coli*. Kelimpahan *E. coli* berjulat antara 3.1 x 10 hingga 1.6 x 10^5 CFU 100 mL^{-1}, dan jumlah pepejal terampai berkorelasi secara signifikan dengan kelimpahan *E. coli* ($r^2 = 0.165$, $p < 0.001$) dan hujan ($r^2 = 0.342$, $p < 0.001$). Kumpulan filogenetik B1 (29.7%) dan A (29.4%) adalah yang paling lazim sedangkan kumpulan B2 dan D kurang lazim. Kelimpahan kumpulan filogenetik D yang lebih tinggi di hulu Sungai Kelantan menunjukkan pencemaran feses terutamanya berasal dari haiwan. Analisis korelasi kanonik menunjukkan bahawa kumpulan filogenetik B2 berkembang pesat di dalam air dengan paras oksigen terlarut yang lebih tinggi sedangkan kumpulan filogenetik A dan D lebih banyak di perairan dengan nutrien anorganik yang lebih tinggi, misalnya NH_4, NO_2 dan NO_3 manakala kumpulan filogenetik B1 kelihatan mempunyai toleransi kemasinan yang lebih baik di kalangan kumpulan filogenetik.

Kata kunci: *E. coli,* pencemaran feses, Sungai Kelantan, kumpulan filogenetik

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A/E	:	Attaching and effacing
IMWQS	:	Interim Marine Water Quality Standards
USEPA	:	United States Environmental Protection Agency
LEE	:	Locus of enterocyte effacement
TSS	:	Total suspended solids
DO	:	Dissolved oxygen
Rpm	:	Rotation per minute
FIB	:	Fecal indicator bacteria
ASi	:	Amorphous silica
AIDS	:	Acquired immunodeficient syndrome
NWQS	:	National Water Quality Standards
PCR	:	Polymerase Chain Reaction
VBNC	:	Viable but not culturable
EAF	:	EPEC adherence factor
POM	:	Particulate organic matter
CFU	:	Colony forming unit
CV	:	Coefficient of variation
EPS	:	Extracellular polymeric substances
BSi	:	Biogenic particulate silica

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CHAPTER 1 : INTRODUCTION

Escherichia coli (*E. coli*) is the subgroup of coliform that is commonly found in the intestine of both human and animal. It has been used as an indicator for fecal contamination in the aquatic system and as an indicator of food hygiene and food safety (Yucel & Ulusoy, 2006). Most of the *E. coli* strains are harmless but some pathogenic strains are responsible for gastrointestinal and extraintestinal infections in humans. Examples of extraintestinal infections caused are urinary tract infection, nosocomial bacteremia and myositis (Russo & Johnson, 2003; Galvin et al., 2010).

Pathogenic *E. coli* that causes extraintestinal infection are enteropathogenic *E. coli* (EPEC), enterohaermorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Hamelin et al.., 2007). EPEC is categorized as attaching and effacing (A/E) pathogens associated with infantile diarrhea (Ochoa & Contreras, 2011) back in the 1940s but eventually became widespread in developing countries (Croxen et al., 2013). EPEC is transmitted through the fecal-oral route and develops lesions on intestinal epithelial cells. Similarly, EHEC also forms A/E lesions but genetically differ from EPEC (Kang *et al*., 2018). Serotype O157:H7 is the most common pathogen from EHEC group causing hemorrhagic colitis (Riley et al., 1983) and has been reported in numerous outbreaks in the United States (Croxen et al., 2013). EIEC is an extremely invasive pathogen and is highly adaptive to different environments during the course of invasion (Marteyn et al., 2012). Symptoms of EIEC infection include mild watery diarrhea and fever. EAEC and ETEC are known to be the causative agents for traveler's diarrhea. Symptoms of EAEC infection include watery diarrhea, abdominal pain, mild fever and nausea (Croxen et al., 2013). EAEC could also causes malnutrition in children as intestinal epithelium is damaged by mucosal inflammation (Estrada-Garcia & Navarro-Garcia, 2012). On the other hand, ETEC is determined by the ability to synthesize heat

labile (LT) and heat stable (ST) enterotoxin. DAEC is a pathogen that is not categorized into a normal pattern of localized adherent or A/E and causes persistent diarrhea in young children (Kang *et al*., 2018). It is defined by its ability to adhere to epithelial HEp-2 cell in a scattered pattern (Thomazini *et al*., 2011).

E. coli can be divided into four phylogroups, namely phylogroup A, B1, B2 and D based on the presence or absence of three genes, i) *chuA*, gene required for hemo transport in EHEC; ii) *jyaA*, unknown function gene; and iii) TSPE4.C2, anonymous DNA fragment (Clermont et al., 2000). Most commensal *E. coli* are derived from phylogroup A and B1 and vary widely in their capacity to persist in the human intestine. Obligatory pathogens responsible for acute and severe diarrhea (EHEC, ETEC and EIEC) are also found within these groups. Most virulent extraintestinal strains belong to group B2 and D (Luna et al., 2010) while the pathotypes linked to chronic and mild diarrhea (EPEC, EAEC and diffusely adherent *E. coli*) is distributed across all the four phylogenetic groups. Studies have shown that *E. coli* group A and B1 are more persistent in the environment and frequently recover from aquatic environments (Berthe et al., 2013). Furthermore, *E. coli* group A and B1 have been recognized as emerging intestinal strains (Escobar-Paramo et al*.,* 2006) and closely associated with antibiotics resistance.

There is an increasing concern in pollution of river water systems in Malaysia which poses serious risk of health to public (Camara et al., 2019). River water plays a major role in agriculture, fishery, logging and manufacturing industry (Pooveneswary et al., 2020). Local communities residing along rivers rely on river water for water supply and contributes to anthropogenic pollution (Dasar et al., 2009). Rivers in Malaysia are sometimes used as heavy metal dump sites either legally or illegally. Some contaminants in rivers will be retained in water or taken up by organisms and ultimately affect humans as the final consumer (Dasar et al., 2009).

The Department of Environment Malaysia (DOE) has used water quality index (WQI) to evaluate the status of river water quality. WQI is computed based on six main parameters: biochemical oxygen demand (BOD), dissolved oxygen (DO), chemical oxygen demand (COD), suspended solids (SS), ammonia nitrogen (AN) and pH. The WQI serves as the basic for river assessment in relation to pollution categorization, health risk and the designation of classes of beneficial uses as provider under the Interim National Water Quality Standards (INWQS) for Malaysia. The assessment of the WQI conducted by the DOE is physio chemical based; it does not include coliform based indicators. Furthermore, the WQI is used to describe the water quality conditions at specific locations and time therefore the information could be subjective and biased especially in sanitary inspections and risk matrices to quantitative water quality risks.

Kelantan River is the main river which is the confluence of Galas river and Lebir river that runs along Kelantan State. It has a catchment area of 12,000 km^2. The river passed through four major towns namely Kuala Krai, Tumpat, Pasir Mas and Kota Bharu (Pradhan & Youssef, 2011) before discharging into the South China Sea. The Kelantan River serves as an important water source for the local people for domestic uses, transportation, agriculture, irrigation fish industries and sand mining. Previous studies have shown that the *E. coli* and total coliform are frequently found in the Kelantan River at concentrations that exceed limits for Interim Marine Water Quality Standard (INWQS) and Water Quality Index (WQI) (Rathi et al., 2009; Ngah et al., 2012; Basri et al., 2015; Bamaiyi et al., 2017) which led to higher incident rate of food and water borne diseases in Kelantan compared to other states. Being in the Monsoon belt, incidence of flood in Kelantan is frequent and high, which can overwhelm sanitation systems and stir up rivers, increase volume and occurrence of *E. coli* and total coliform between the Kelantan River Delta and its adjacent coastal waters. As marine and river pollution are interrelated.

In Malaysia, *E. coli* has been identified as one of the main contaminants of the coastal environment. Nowadays, marine pollution has been regarded as a global environmental issue in particular for countries with coastal zones. Although a surveillance program is employed in Malaysia to monitor marine water quality, public recreational beaches are left out of the scheme (Ahmad et al., 2013). Recent studies have shown the presence of *E. coli* in water of some popular recreational beaches in Peninsular Malaysia and the numbers detected have exceeded the quality threshold for recreational marine water (Hamzah et al., 2011; Gasim et al. 2015). Subsequently, pose substantial human health risk either to individuals who have direct contact with polluted water or consumption of tainted seafood. The prevalence of *E. coli* in coastal Malaysia could be attributed to the marine pollution coming from land-based activities such as wastewater, solid waste, agricultural runoff (BOBLME, 2011) through rivers. Unfortunately, to date many Malaysian beaches are not well monitored, managed and assessed by local authorities.

There is growing concern about the occurrence *E. coli* in aquatic environments. Indeed, to date the population structure and virulence factors of *E. coli* in tropical aquatic ecosystems still remains unknown. Although studies have been conducted on water quality monitoring and characterization of *E. coli* from selected waterways in Kelantan. Further study is needed to determine the abundance, genetic diversity and virulence factors of *E. coli* in surface water of Kelantan River and its adjacent coastal waters in order to have better understanding on *E. coli* epidemiology, transmission and ecology. Therefore, this study aimed to i) determine the abundance of *E. coli* in Kelantan River Delta and its adjacent coastal waters; and ii) investigate the genetic diversity and virulence factors of *E. coli* isolated from Kelantan River and its adjacent coastal water.

CHAPTER 2 : LITERATURE REVIEW

2.1 Enterobacteriaceae

Enterobacteriaceae is a family of facultatively anaerobic gram negative rod shaped bacteria, non-sporing, motile with peritrichous flagella (except *Shigella* and *Klebsiella*) and non-capsulated (except *Klebsiella*) form. They ferment glucose, reduce nitrates and are oxidase negative. They are ubiquitous in the environment (e.g. soil, water, plants) and members of this family include some of the normal inhabitants in the gastrointestinal tract of animals and humans (Jones, 1988; Forsythe *et al*., 2015). There are 51 genera and 238 species in this family but >95% of clinically significant strains fall into 10 genera and <25 species. Genera under this family are *Citrobacter, Enterobacter, Escherichia, Klebsiella, Salmonella and Shigella*. Enterobacteriaceae has widespread distribution and comprises opportunistic pathogens and disease-causing potential bacteria that can cause morbidity and mortality. The increment of multidrug resistant Enterobacteriaceae in recent decades has raised global public health concern (Schwaber & Carmeli, 2008; Roca et al, 2015).

2.2 Coliform

Coliform can be divided into total coliforms or fecal coliforms. According to Mishra *et al* (2018), total coliform consists of both soil intermediates and fecal forms while fecal coliforms are originated from intestinal tract of warm-blooded animals. They are also used as standard indicators for water quality.

Total coliform is a subset of Enterobacteriaceae group, they are facultative anaerobic, rod shaped, gram negative and ferment lactose with production of gas (Bitton, 2005). Total coliform is found in high concentration from mammals' discharge. *Serratia*, *Escherichia coli*, *Enterobacter*, *Citrobacter* and *Klebsiella* belong to the total coliform group. They are useful indicator in the determination of recreational water and potable

water quality due to the fact that they are less sensitive compared to viruses and protozoan cysts to changes in environmental conditions (Rochelle-Newall et al., 2015). Meanwhile, fecal coliform is a subset of total coliform which consists of bacteria like *E. coli* and other thermotolerant coliforms that ferment lactose at 44.5°C (Cabral, 2010). The detection of fecal coliform shows the presence of contamination by mammals.

2.3 *Escherichia coli*

The human intestine consists of more than 350 species of commensal microorganisms living in harmony. In one gram of feces from a healthy human being, a concentration of 10^{12} organisms can be found and represents a balanced ecosystem (Hooper & Gordon, 2001). A number of genera from the family Enterobacteriaceae can be found in healthy and sick animals. Among them are *E. coli* which is one of the most important bacteria that generally present more than one million (10^6) cells in one gram of feces and are frequently released into the environment (Ishii & Sadowsky, 2008). Although they are normal colonists within the intestinal tract, they are commonly associated with human diseases.

E. coli are gram negative, rod shaped bacterium under the family of Enterobacteriaceae. It is well adapted to its habitat and is able to grow with glucose as the only organic source (Hooper & Gordon, 2001). *E. coli* can grow in the presence and absence of oxygen (O_2). Under the presence of O_2, *E. coli* can survive by means of fermentation or anaerobic respiration. Most of them are not pathogenic but in fact, beneficial to their host. However, there are small populations of *E. coli* which can cause diseases and STEC O157:H7 *E. coli* was identified when two observations were made. The first observations would be bloody diarrhea and severe abdominal cramps which is termed as hemorrhagic colitis (HC). The second observation was irregular reports on hemolytic uremic syndrome (HUS) with fecal cytotoxin and also *E. coli* in stools (Grisaru, 2014). *E. coli* that causes diarrhea

can be distributed into six common categories which are enteropathogenic (EPEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), diffusely adherent (DAEC) and enterohemorrhagic (EHEC). All these groups can be sorted according to their serotypes and pathogenesis (Chandra *et al*., 2013).

2.4 Diversity of *E. coli*

E. coli can be categorized phenotypically and genotypically. A simple and traditional way of categorizing is based on Kauffman classification scheme (Kang *et al*., 2018). The three antigens involved are somatic (O), capsular (K) and flagellar (H). *E. coli* strains can also be categorized based on their phenotypic characteristics such as the presence of flagellar, ability to form biofilm and ability to utilize carbon compounds (Ishii & Sadowsky, 2008). The persistence of *E. coli* B1 in the environment has been suggested to be the ability to utilize aromatic compounds and formation of biofilm (Berthe et al*.,* 2013). *E. coli* can also be categorized based on their phylogenetic characterization into four phylogroups namely phylogroup A, B1, B2 and D by detecting on the existence or absence of three genes: i) *ChuA*, gene involved in heme transport; ii) *YjaA*, gene of unknown function and iii) TspE4.C2, an anonymous DNA fragment (Clermont et al*.,* 2000). Most commensal *E. coli* are derived from phylogroup A and B1 (Walk et al*.*, 2007; Mushtaq et al*.*, 2011). Obligatory *E. coli* responsible for acute and severe diarrhea (EHEC, ETEC and EIEC) are also found within these groups. Strains belonging to group A are predominantly found in the human intestine whereas B1 in animals. The B2 and D are closely associated with extraintestinal infections (Gordon et al*.*, 2005; Jaureguy et al., 2008).

The phylogroup combination of A and B1 is commonly detected in non-human mammals, the phylogroup combination of A and B2 are being prevalent in humans (Escobar-Páramo et al., 2006). The abundance and distribution of *E. coli* phylogroups in

mammalian hosts depends on climate, host diet, and body mass. Phylogroup A and B1 can be isolated from any vertebrate group. Phylogenetic group B1 tends to reside in birds and carnivorous mammals with tube-like gastrointestinal tract. Phylogroups B2 and D are mostly restricted to endothermic vertebrates. The predominant of Phylogroup B2 in omnivorous and herbivorous animals are found mainly in caecum (Gordon & Cowling, 2003).

The resident and transient commensal *E. coli* strains that are present in humans can be differentiated based on their ability to persist in the intestines. Aerobactin production, P-fimbriae, capsule are some of the major virulence determinants that enable *E. coli* strains to persist in human intestines (Nowrouzian et al., 2005). Studies have shown that phylogenetic group B2 generally harbours more virulence factors compared to phylogroups A, B1, and D (Picard et al. 1999; Nowrouzian et al., 2005).

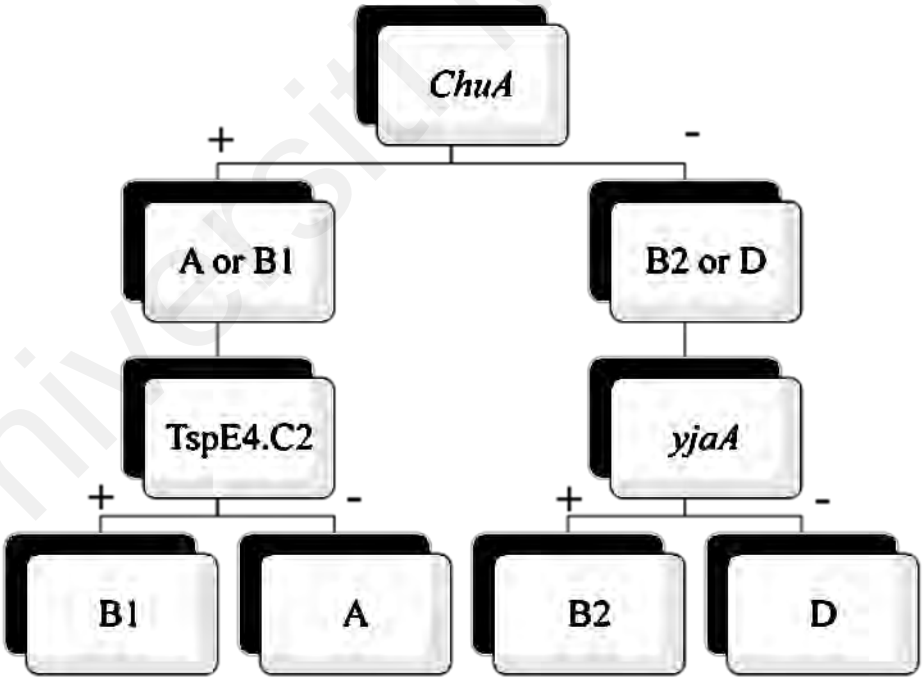

Figure 2.1 : Dichotomous decision in characterizing *E. coli* phylogenetic group (Clermont et al., 2000)

2.5 *E. coli* in Environment

E. coli has been assumed to have poor survivability in natural environment due to various environmental factors, including temperature (Williams et al., 2005; Ishii et al.,

2006), humidity (Solo-Gabriele et al., 2000; Desmarais et al., 2002; Byappanahalli et al., 2004), salinity, pH, solar radiation, predation and etc (Bretta & Höfle, 1992; Byappanahalli et al., 2004; Solo-Gabriele et al., 2000). However, recent studies have shown that *E. coli* are able to survive in tropical environments (Byappanahalli et al., 2004, 2006). Warm water and high concentrations of nutrients in tropical waters creates favorable conditions for *E. coli* to survive and replicate. A study conducted by Na et al. (2006) showed that *E. coli* entered viable-but-nonculturable (VNBC) state when culturing medium was held at 4°C. Byappanahalli et al. (2006) isolated *E. coli* strains from exclosure protected forest sediments and Ishii et al. (2006) also isolated *E. coli* from temperate soil samples. Both strains are genetically similar and DNA fingerprints showed distinct patterns with animal-borne *E. coli*. The use of simple carbon compounds and nitrogen sources by *E. coli* enables it to grow in the environment. The ability to utilize sucrose and various aromatic compounds provide an advantage in the environment (Berthe et al., 2013). With the observation above, some proposed that *E. coli* is a "naturalized" member of the environment.

2.6 *E. coli* as pathogen

E. coli can be harmless but some strains are able to cause various extraintestinal and intestinal diseases through the use of virulence factors (Kaper et al., 2004). They are characterized based on their specific virulence factors which include adhesins, invasins, toxins and capsule. (Fairbrother & Nadeau, 2006). There are at least six *E. coli* pathotypes have been defined based on: virulence factors, clinical diseases and phylogenetic profile.

i) ETEC (Enterotoxigenic *E. coli*) is the main cause of traveler's diarrhea and infantile gastroenteritis. Prevalence of ETEC in developed countries is not high as compared to developing countries where clean water is not readily available. Children below the age of five are mostly the victims of ETEC, symptoms of

diarrhea caused by ETEC are profuse watery diarrhea which will lead to malnutrition and dehydration. A proteinaceous surface polymer called colonization factors (CFs) were produced by ETEC to facilitate adherence to intestinal mucosa and induce diarrhea in animals and humans. (Isidean et al., 2011). Heat labile enterotoxin (LT) and heat stable enterotoxin (ST) are the virulence factors of ETEC. Diarrhea may induced by toxins independently or both at the same time (Nazarian et al., 2012). LT and ST induce its toxin effect by causing phosphorylation and stimulates chloride channel, follows by discharged of electrolytes and water, resulting in diarrhea (Mirhoseini et al., 2018).

ii) EPEC (Enteropathogenic *E. coli*) was the first to be identified among the diarrheagenic *E. coli* and associated with infantile diarrhea epidemic in 1940s and 1950s (Hu & Torres, 2015) and appeared to be the most common pathogens. EPEC could be isolated from 40% infants with acute diarrhea (Fagundes-Neto & Scaletsky, 2000). EPEC belongs to a group of bacteria which has the ability to form attaching and effacing (A/E) lesions on the surface of intestinal epithelial cell which is sometimes referred to as localized adherence. EPEC inserts its own Tir receptor to the host cell which is then phosphorylated on tyrosine and serves as receptor for intimin (McWilliams & Torres, 2015). According to Trabulsi et al. (2002), typical EPEC produce virulence factors encoded in locus of enterocyte effacement (LEE) and EPEC adherence factor (EAF) plasmid. However, for atypical EPEC, they lost EAF plasmid and may be less virulent than typical EPEC. These pathogenic strains of *E. coli* are also called Shiga-like toxin producing *E. coli* (STEC) and can produce potentially harmful toxin called Shiga toxin 1 and 2 (*stx*1 and *stx*2) because the toxins are similar to toxins produced by *Shigella dysentriae.* STEC is also known as Verotoxin producing *E. coli* (VTEC) as the

toxin (*vtx1* and *vtx2*) produces acts on Vero cell. The function of *stx1* and *stx2* is to disrupt the protein synthesis in the host.

iii) EAEC (Enteroaggregative *E. coli*) was described by Nataro et al. in 1987 for their ability to adhere to Hep-2 cell in a brick like pattern (Johnson & Nolan, 2009). In developing countries, EAEC is associated with persistent diarrhea and being found to be the cause of irregular diarrhea in AIDS patients (Sousa, 2006). Transmission route for EAEC is through fecal-oral whereby contamination of food and water plays a major role (Cennimo et al., 2007). Virulence factor for EAEC is aggR which involves expression of adherence factor, dispersin protein and cluster of genes. Pathogenesis of EAEC encompasses 3 stages: i) adherence to intestinal mucosa by adherence factor and aggregative adherence fimbriae; ii) production of mucus and biofilm on intestinal villi and iii) release of toxins and generate inflammatory response, intestinal secretion and mucosal toxicity which are destructive to intestinal villi's tips and sides (Harrington et al., 2005; Nataro, 2005). Identification of EAEC is through Hep-2-cell-adherence assay. This assay identifies "stacked brick" aggregative phenotype and is gold standard in diagnosis (Huang et al., 2006).

iv) EIEC (Enteroinvasive *E. coli*) are characterized based on their ability to infect HeLa cells. EIEC can invade the mucosa of the human colon which will result in watery diarrhea with blood. However, some patients might develop abdominal cramping, fever, and bloody mucoid stools. EIEC invade microvilli, multiply in cytoplasm and move to adjacent epithelial cells. This will cause localized inflammation, ulceration and diarrhea (Liévin-Le Moal & Servin, 2013). The ability to invade host cells is due to the possession of Inv plasmid. Distinct

chromosomal and plasmid-borne virulence genes are required for cellular invasion which is similar to Shigella spp.

v) EHEC (Enterohaemorrhagic *E. coli*) is the subset of STEC because EHEC produces Shiga toxin. Intimin, a protein encoded by the gene *eae* which gives EHEC the abilities to form A/E lesion in the intestinal wall by disrupting the brush border microvilli (O'Sullivan et al., 2005). The gene is located at LEE. EHEC is also one of the main concerns in the outbreaks of HC and HUS. HC is characterized by bloody diarrhea, abdominal cramps, inflammation of large bowels and vomiting (Madic et al., 2011). HUS followed HC which is characterized by the onset of low platelet count and renal injury and death which include seizures and coma (Razzaq, 2006). The main reservoir for EHEC is cattle, in particular, and also other ruminants (Meng et al., 2012). Raw meat and unpasteurized milk are also one of the sources for EHEC according to Rahimi *et al* (2012). The route of transmission for EHEC is through contact with water while swimming in contaminated lakes or pools. Transmission also occurs through person to person and direct contact with animal fecal through recreational activities (O'Sullivan et al., 2005).

vi) DAEC (Diffusely adhering *E. coli*) is characterized by their ability to form a diffuse adherence (DA) pattern on cultured HeLa cells (Servin, 2005). There are two different adhering patterns, one of the patterns is DAEC was seen around or covered the whole surface of HeLa cells while the other pattern is DAEC was seen to bind to a localized area which formed microcolonies (Thomazini *et al*., 2011). The first pattern is called diffused adherence while the latter is called localized adherence. DAEC secretes adhesins which triggers intestinal cell lesions and

injuries. The infection damages activities of villi on the intestinal wall (Kaper et al., 2004).

2.7 Kelantan River Basin and its Adjacent Coastal Water

Kelantan is one of the states in Malaysia having the largest river and consists of mountainous terrain. It is located at the north eastern region of Peninsular Malaysia. Southern part of Kelantan state has more mountainous terrain, Titiwangsa Mountain Range can also be found at the southern part, with coastal beach to the north and east. About 95% of Kelantan River basin is mountainous and only 5% is lowland plains. (Wang *et al*., 2017; Milliman & Farnsworth, 2011). Mangrove and coastline can be seen within braided estuary, sand bar found at the estuary ranges from 0.4 to 0.8 km in length (Wang *et al*., 2017). Sandy beach can be seen along Kelantan River mouth with width of 20 to 150 m. Whereas the ocean floor sloop gently with water depth ranges from 50 to 2500 cm (Radzir *et al*., 2016).

Kelantan River is among the rivers in Malaysia that affected by flood events the most. This is due to significant changes in land use, urbanization and inefficiency in development, coupled with meteorological factors. From year 1970 to 2000, more lands close to Kelantan River are being modified for agriculture purposes and also deforestation (Saadatkhah *et al*., 2016). The upstream of Kelantan River mainly surrounded by tropical rainforests while agriculture crops (rice field, palm oil and rubber) are mainly concentrated in mid to downstream. In year 2010, lands around Temangan, Machang and Kota Bharu were being exploited for new manufacturing plant and agricultural activities (rice field, palm oil, etc). More lands were being used after year 2013 and also deforestation (logging), particularly at Kuala Krai in year 2017 (Fitri *et al*., 2021). Due to development of urban area, it was found that Kelantan River is polluted with heavy metal and coliforms (Yen & Rohasliney, 2013; Ab Razak *et al*., 2016). These pollutions can in

turn affect the health of residents through enrichment in vegetable crops and aquaculture products (Hashim *et al*., 2014; Khairiah *et al*., 2014). There are 10 main rivers in Kelantan state, namely Sungai Nenggiri, Sungai Lebir, Sungai Galas, Sungai Pergau, Sungai Kelantan, Sungai Golok, Sungai Kemasin, Sungai Pengkalan Chepa, Sungai Pengkalan Datu and Sungai Semerak. The 2 branches commonly known which constitute Kelantan River, namely Galas River and Lebir River. Galas River make up 54% of the total Kelantan River basin while Lebir River makes up 17%. The river emerges from southern uneven and steep region with elevation of 1km to 2km. Sungai Nenggiri located at the south west region flows towards north east direction and converge with Sungai Galas at Bertam. Sungai Galas then flows toward Jeli, Dabong and converge with Sungai Pergau. Sungai Lebir from Gunung Gagau flows towards north west direction and meet with Sungai Galas, which flows towards north east, at Kuala Krai. The river is known as Sungai Kelantan from Kuala Krai onwards which flows north for approximately 100km towards river mouth, passing through Kota Bharu. Kelantan River basin is less uneven moving towards the central region of basin where mostly consist of low hills and elevation less than 7500cm. High lands are situated at western, southern and eastern part of river with elevation of 1km to 1.5km. The upland is covered with virgin jungle while rice field, oil palm and rubber are grown at lowlands (Osman *et al*., 2018).

Kelantan River is the main river that runs along Kelantan state which is the confluence of Galas river and Lebir river and moving towards the South China Sea. The river passed through four major towns like Kuala Krai, Tumpat, Pasir Mas, and Kota Bharu before entering the South China Sea (Pradhan & Youssef, 2011). Kelantan River supports a catchment of 13100 km^2 and is dominated by sedentary soils and alluvial soils (Adnan & Atkinson, 2011). Northeast monsoon season (November – January) affects the river flow with a maximum annual rainfall of 1750 mm. Receiving end of Kelantan River is South China sea whereby coastal water is a valuable natural resource serving multi purposes,

e.g. for marine recreation, aquaculture and recipient receiving wastewaters. Besides that, the Kelantan River also serves as an important water source for local residents. Roles of Kelantan River are as a mode of transportation, as water source for crop irrigations, aquaculture industries, sand mining and domestic use. There are about 128 sand mining operations along the Kelantan River which causes the river water to turn turbid since the early 1990s.

Kelantan state comprises of 956,139.9 ha of Jungle and forest reserve which is about 63.66% of the total area (Satyanarayana *et al*., 2011). In the upstream of Kelantan basin, agricultural area covers 395,156.8 ha (26.32%) of the total area which urban area consists of only 14,616.5 ha (0.92%). In 2018, agriculture farming was introduced at Lojing Highland in Gua Musang and has brought about disagreement in residents due to large amount of silt and clay runoff into the river (Osman *et al*., 2018).

The climate in Malaysia is described as uniform temperature, humid and abundant of rainfall. Malaysia also experienced both southwest (SW) and northeast (NE) monsoon season. Kelantan state is governed by NE (November to March) and SW monsoon (May to September) and frequent rainfall. Highest rainfall is also observed during the inter-monsoon season (April to May and October to November), which is the transition between SW and NE monsoon. Driest month in the year falls on January.

The coastal area around Kelantan state is prone to flooding and this problem has affected the social economy development (Osman *et al*., 2018). In year 1990, Kelantan faced a big flood on November and December with rainfall recorded from 23rd to 28th November 1990. A total of 203.2mm and 1159mm of rainfall water was recorded for coastal and rural area respectively. Water level reached a dangerous level of 26.16m on 13th December 1990 (Osman *et al.*, 2018). Villages in Kuala Krai were flooded for 2 days with level ranging from 30cm to 140cm while water level at Jambatan Guillemard has exceeded the danger level 16m.

Kelantan was faced with another severe and serious flood in middle of December 2001. The level of rainfall recorded was more substantial than before, a total of 3064.17mm equivalent to 113.49% was documented. Water level recorded at Kuala Krai has exceeded the danger level of 25m on 24TH December 2001. The flood has affected major townships including Gua Musang, Kuala Krai, Tanah Merah, Kota Bharu, Pasir Mas and Tumpat. In 2004, high water level was recorded from Kelantan River. Areas that are close to the river were affected. Water level recorded at Tambatan Di Raja was 2m above the danger level on 13th December 2004. River water overflowed at Tanah Merah due to heavy rain and caused 4m depth flooding to nearby area (Cekok Ipoh and Kg. Kulim) (Osman *et al*., 2018).

The most recent flood is most severe flood in decades at the end of December 2014. Continuous heavy rain occurred between 17 December 2014 to 3rd January 2015 whereby residents near to the river basin had to evacuate to safety. About 330,000 residents were affected and estimated RM50 million worth of loss has been recorded. Nearly 10 inches of rainfall were recorded between 21 December to 22 December 2013 which further aggravated the flood situation (Saadatkhah *et al*., 2016). In addition to rainfall, high tide affected by the moon was also one of the factors for heavy flood. Major townships along Kelantan River (Kota Bharu, Pasir Mas, Kuala Krai and Tanah Merah) were badly affected.

Several studies have been carried out on Kelantan River in the past and it was found that the concentration of *E. coli* and total coliform have exceeded the threshold for safe water drinking and usage as compared to the standards given by Interim Marine Water Quality Standard (IMWQS) and Water Quality Index (WQI). The incidence rate of food and water borne diseases in Kelantan have been found to be higher compared to other states (Zain & Naing, 2002). Basri et al. (2015) found that the concentration of *E. coli* was 1.70 x 10^3 CFU/mL. Fauziah et al. (2014) revealed that 49% (221/454) of untreated

groundwater samples were contaminated with total coliform ($3.18 \pm 1.80 \times 10^4$ MPN/100mL) and 14% (65/454) samples were contaminated with *E. coli* (5.09 ± 30.8 MPN/100mL). Ngah et al. (2012) reported that Kelantan beach water has high concentration of *E. coli* (2.03×10^3 MPN/100mL).

2.8 *E. coli* and Factors affecting its Survival

i) Temperature. *E.coli* generally cycle through two different habitat and was thought to have half-life of 2 days. It was first thought that *E. coli* could only exist in intestine of endoderm before excrete into the environment. The first habitat being inside human gut system while the second is environmental habitat (water, sediments and soil). These two habitats differ very much in physical and chemical factors which ultimately affects the survival of *E. coli* where environmental habitat poses greater stress as it is not the original habitat *E. coli* was first found. Conventional thought on limited *E. coli* survival in the environment are (i) new source of fecal matter is require to maintain the number of *E. coli* in the second habitat; (ii) lack of new host colonization. Due to the relationship between population of endoderm and contamination of fecal matter, direct discharge of fecal matter remains the main factor influencing concentration of *E. coli* in environment. A study conducted by Ishii *et al*. (2006) showed that *E. coli* able to survive and proliferate in environment outside host and poses risk of colonizing new host. It may become autochthonous and capable of surviving and proliferate without needing disposition from endoderm. An investigation by Ishii *et al.* (2006) showed that *E. coli* are able to survive up to 32 days in soil sample incubated at 15°C. They also looked into the fingerprinting of *E. coli* a year apart and found 92% similarity which shows that *E. coli* strains has become neutralized, which in

this context, means that *E. coli* can integrate into secondary habitat and proliferate in a sufficient rate to maintain its population (Jang *et al*., 2017).

The first and probably most prominent factor that effect *E. coli* in their survival outside host is temperature (Petersen & Hubbart, 2020b). The primary habitat, which is the intestinal gut, had relatively stable temperature of 37°C while the secondary habitat, which is the environmental water, sediments and soil, can have temperature range greatly from below freezing to tropical temperature (depending on regions). *E. coli* can grow in soil which has temperature of more than 30°C but their death rate is also higher than in cold environment (<15°C) (Ishii *et al*., 2009). An experiment conducted by Whitman *et al*. (2003), showed that *E. coli* can survived on dried algal in airtight plastic bags at 4°C for over 6 months. In addition, the environment in intestinal gut represents anaerobic environment whereas the environment water, soil and sediments are a mixture of anaerobic and aerobic. When present in the environment habitat, serotype like O157:H7 can pose significant danger to human if accidentally ingest.

According to Van Elsas (2011), *E. coli* generally experienced biphasic life throughout their evolutionary lifetime. The biphasic life cycle consists of host-dependent and host-associated phase. Depending on host type, the interaction between *E. coli* and host may differ and evolution in *E. coli* genomic island should also be taken into consideration. For example, *E. coli* that were ingested by host have to go through severe stress like extremely low pH in the digestive tract. Environment inside digestive tract is more constant and is more favourable for growth and survival. As compared to open environment, carbon or glucose availability, osmolarity, temperature, aerobic or anaerobic conditions fluctuate and ranges widely. In order to survive the challenging environment, *E. coli* acquire evolution to its genome to enable them to uptake amino acid and sugars through

ABC transporters or siderophore-mediated iron uptake system (Schubert *et al*., 2004). This shows that *E. coli* are able to survive in open environment by utilizing various nutrients (Ihssen *et al*., 2007).

When encountering low temperature, *E. coli* developed mechanism which involved modifying membrane to maintain membrane fluidity and maintain structural integrity of macromolecule and macromolecular assemblies (Berry and Foegeding, 1997). The composition of membrane lipid is changed to shorter lipid chain or unsaturated fatty acid as growth temperature decreased, this can maintain the fluidity of the membrane. The uptake of nutrient and ion pumping by intrinsic protein is being controlled as well. An increased in cis-vaccenic acid (C18:1) over palmitic (C16:0) was also observed in *E. coli* at low temperature. These changes aids in increasing membrane fluidity as well.

Cold shock proteins (CSPs) is one of the most studied responses to low temperature. Microorganisms starts to synthesize smaller proteins (7 kDa) in increasing amount during change to colder temperature. For *E. coli*, about 15 different cold shock proteins are being synthesized, which are involved in transcription, translation, mRNA degradation, protein synthesis, and recombination in *E. coli* (Graumann and Marahiel, 1998). Goldstein *et al*. (1990) found that cold shock proteins shared a 45% similarity with DNA of food-borne spoilage and pathogenic Gram-positive and Gram-negative bacteria.

Cold shock protein A (CspA) is the main shock protein synthesized by pathogenic and commensal *E. coli*. CspA has low binding affinity and are able to bind to RNA without any specific sequence (Jiang *et al*., 1997). CspA in *E. coli* function to destabilized the secondary structure of RNA, making mRNA more susceptible to RNase degradation. Therefore, it act as an RNA chaperone and helps in translation at low temperature. Expression of CspA at lower temperature was regulated at the

level of transcription and translation. Proper folding of proteins and refolding of cold damaged proteins after cold shock is essential, as well as heat shock (Kandror and Goldberg, 1997). For heat shock protein, they act as chaperone for protein in high temperature whereas cold shock protein act as chaperone for RNA.

ii) Hydrological conditions. An increase in pollutants in water bodies can be associated with increase precipitations and runoff from stormwater event, which can result in increased turbidity, total suspended solids concentration, organic matters and faecal contaminations (Wu *et al*., 2016; Rochelle-Newall *et al*., 2016). Identically, increased in *E. coli* concentration relative to based flow condition is also correspond to increased overland and streamflow during storm events (Ribolzi *et al*., 2011; Ekklesia *et al*., 2015).

According to Knierim *et al*. (2015), the magnitude of increase in *E. coli* concentration is 15-fold and Rochelle-Newall *et al.* (2016) has reported at 1000-fold. Rochelle-Newall *et al*. (2016) also describe the factors that affect *E. coli* concentration in during storm events. These factors include type of manure used in upland agriculture, rainfall intensity, rainfall duration, type and age of faecal disposition and *E. coli* adsorption to soil particles. Based on these factors, Rochelle-Newall *et al*. (2016) came out with a formula to calculate concentration, which is $C_s \geq C_0 l_s$ where C_s is *E. coli* concentration during storm events and C_0 is base flow *E. coli* concentration. l_s is the coefficient of increase range from 15.8283 to 1000 which changes based on the above factors.

For example, contribution from ground water to streamflow generally has a lower concentration of *E. coli* due to the fact that ground water typically has lower *E. coli* concentration and also lower concentration were received during storm events (Rochelle-Newall *et al*., 2016). Although ground water decreases the

concentration of *E. coli*, it increases shear stress of river bank, causes resuspension of sediments and promote cell proliferation. According to Ribolzi *et al*. (2016), approximately 11% of the total *E. coli* concentration found in river water during storm event was contributed by sediments resuspension.

Pandey and Soupir (2013) came out with a formula to calculate concentration of *E. coli* that were resuspended during storm events, the formula is $R_0 = C_S \times E_0(\frac{\tau_b - \tau_{cn}}{t_c - t_{cn}})^{n_a}$, where R_0 represents resuspended *E. coli* (CFU/m^{2}S); E_0 represents erosion rate (cm/s); τ_{cn} is critical shear stress (N/m^2) of non-cohesive particles while τ_c is the critical shear stess (N/m^2) of cohesive particles, and n_a is the particle size. Value of τ_b can be obtained by multiplying specific gravity of water (N/m^3) , hydraulic radius (m) and water surface slope (m/m). Meanwhile τ_{cn} can be calculated using the formula $\tau_{cn} = d(4.14) \times 10^3$, where d is the particle size (m). According to Weiskel *et al*. (1996), Lick's equation can be used to calculate τ_c. The formula is $\tau_c = \tau_{cn}(\frac{1 + ae^{b_c p^\delta}}{d^2})$, where a and b_c are constants of 8.5 x 10^{-16} and 9.07 cm^3/g, respectively, p is water density and d is particle size. Wilkinson *et al*. (2006) discussed about 3 resuspension mechanism during high flows. The first mechanisms is steep strong wave (due to influx of water during precipitation period), where height of wave is higher than water depth. This can cause turbulent wave and lift microorganism from sediments into water body (Wilkinson *et al*., 2006). Second mechanism is less steep wave which can resuspend microorganism but not able to retain in the wave current. Lastly, the third mechanism is high flow turbulence where erosion of water bed or water bank can maintain concentration of microorganism as compared to lower flow.

iii) Water chemistry. Due to the fact that not much studies have been conducted on effects of water chemistry on *E. coli* concentration in the environment, results

from laboratory are being used to extrapolate and simulate to determine the effect of water chemistry on *E. coli* concentration. However, the inclusion of temperature as an independent variable in addition to water chemistry being investigated has skewed (51). Studies conducted by (16,49,50,51) have shown that temperature plays a big role on water chemistry, for example *E. coli* can survive in lower pH at higher temperature. In addition, 51 also showed that pH, lactic acid, and water activity also influence *E. coli* growth. In this context, water activity means the partial vapor pressure of water in substance divide by standard partial vapor pressure of water. Minimal growth of *E. coli* was observed for water activity with value of 0.975 to 0.985, temperature of more than 25°C and pH at 4. On the contrary, a decrease in temperature will raise the pH slightly (49). 59 stated that the optimum pH for *E. coli* growth is between 5 and 7, whereas an increase in acidity or alkalinity decreases survival rate of *E. coli*. Growth limit for *E. coli* in pH is approximately 4, however *E. coli* can stay at pH 2 or 3 in stationary phase. Stationary phase in this context means that number of viable cell remains the same (53). An equation was proposed by 49 to estimate the growth rate of *E. coli* based on value of pH, $rate = (c \times 10^{-pH_{min}})(\frac{10^{-pH_{min}} - 10^{-pH}}{10^{pH_{min}}})$, where rate is the growth rate of *E. coli*, c is constant proportionality, pH_{min} is minimum growth of *E. coli* at tolerable pH, pH is ambient pH. Comparable equation has been constructed for *E. coli* growth with other chemistry variable. For example, the effect of organic acid on *E. coli* growth can be applied on the equation, $rate = c' \times (C_{min} - C)$ where c' is proportionality constant, C_{min} theoretical minimum growth inhibitory concentration of organic acid and C is concentration of organic acid measured. The equation was generated based on the growth *E. coli* that is proportionate to concentration of organic acid by which the concentration is less than the inhibition minimum concentration.

Bacteria cell maintain turgor pressure, which has higher internal osmotic pressure than surrounding medium. Therefore, pressure was exerted outward and this forms the mechanical force for cell elongation (Csonka, 1989). In order to prevent shrinkage and plasmolysis, *E. coli* will activate osmoregulation system to maintain internal osmotic pressure. Osmotic stress condition also able to induced Pex protein which will be discussed further below. This is due to osmotic stress condition can mimic other physiological reactions of starvation (Schultz *et al*., 1988). Besides, increased in interal osmolaroty pressure can inhibit DNA replication, nutrient uptake and cell growth (Csonka, 1989). Osmoregulatory system in *E. coli* maintain equilibrium of internal osmotic pressure with environmental pressure (Pichereau *et al*., 2000).

Osmoregulation is achieved in 2 ways, first is de novo synthesis of osmoprotectants, which will then be stored. Secondly, compatible solutes which are small organic molecules (proline, glycine, carnitine, betaine) are being passed through, charged solutes (potassium ions or glutamate) as well (Pichereau *et al*., 2000). Majority of the compatible solutes are present significantly in food, even with reduced water activity, *E. coli* can still survive by accumulating wide range of compatible solutes.

Using specific transporter, *E. coli* accumulate betaine and proline during osmotic stress or synthesis betaine from choline present exogenously (Csonka and Epstein, 1996). Two group of betaine and proline transporter has been reported for *E. coli*. The first transporter was named ProU which activate in absence or presence of low betaine concentration. The other transporter (ProP) was a fundamental system in *E. coli* (Culham *et al*., 1993). ProU is an ATP-driven, binding protein-dependant system while ProP is ion-motive force-driven transporter.

iv) Nutrients and nutrients availability. Survival of *E. coli* in secondary habitat is also supported by availability of nutrients in environment. According to study conducted by (Tate III, 1978), the concentration of *E. coli* in sediments which is rich with nutrients has three times more the population as compared to concentration of *E. coli* in nutrients deficit sandy soils, which explains the importance of nutrients and organic matter in assisting proliferation of *E. coli*. In addition, (Ishii *et al*., 2006) found that the final concentration of soil *E. coli* was mainly regulated by predation or availability of nutrients. This finding was concluded when they recorded decreasing *E. coli* concentration in soils at 30°C and 37°C following initial rapid cell proliferation. Another study conducted by (Ishii *et al*., 2009) also proved that nutrient limitation is affecting *E. coli* growth by growing *E. coli* in minimal growth (M9) medium without carbon and nitrogen and less than one log increase in CFU was observed. Aside from nutrients, *E. coli* proliferation is also affected by soil water potential due to its effect on nutrient and bacterial movement. Study conducted by Ishii *et al*. (2009) showed significant correlation between *E. coli* proliferation rate and soil water potential ($r^2 = 0.70$, $p < 0.001$). Movement of bacteria in soil also affected by soil water potential as low potential (-1.5 or -0.1 MPa) can give rise to insignificant bacterial movement, decrease solute diffusion and reduced nutrient supply (Griffin, 1981). Milne *et al*. (1991) showed that survival of *E. coli* increases significantly with the presence of dissolved nutrients (glucose and peptone). Glucose can change *E. coli* responses to stressors. This is proved by Wu and Klein (1976) who found that after a period of nutrient starvation, *E. coli* has increased response sensitivity to secondary stressors and nutrient availability.

E. coli halt all metabolic activity and growth during nutrient scarcity and carry out starvation related activity like production of enzyme. These consists of

degradative enzymes like lipase, protease, alkaline phosphatase and glutamine synthetase (Siegele and Kolter, 1992). Increased resistance to some environmental stresses like low pH, high temperature is also induced during starvation. A number of morphological and physiological changes occur when *E. coli* encounter depletion in nutrients and in stationary phase.

During stationary phase, *E. coli* cells become smaller and rounder, DNA is condensed and accumulation of storage compound (glycogen and polyphosphate) occurred (Hengge-Aroni, 1996). Changes to fatty acid composition of inner membrane and protein composition of inner and outer membrane also occurred. The major regulator for *E. coli* in stationary phase or during stress period is RpoS and this regulator is known to produce greater resistance.

There are 2 genes encoding starvation proteins have been identified in *E. coli* which are *cst* gene (controlled by carbon starvation) and *pex* gene (controlled by carbon, phosphorus and nitrogen starvation). These genes are also suggested to be intracellular sensor in *E. coli* (Rowbury *et al*., 1999) Postexponential (Pex) protein encoded from *pex* gene plays a significant role in managing starvation stress and also exogenous stress in *E. coli* (Reeve *et al*., 1984). During heat and oxidative stress, several Pex proteins are being encoded and have been shown to have correlation with enchanced thermal and oxidative resistance during starvation period.

v) Land use. Previous studies shown that land use practices (agriculture and urban land use) cause an increase in *E. coli* population (Gotkowska-Plachta, 2016; Petersen *et al*., 2018; Petersen & Hubbart, 2020). The use of manure and density of livestock in agriculture regions is the main reason for increased *E. coli* population (Jamieson *et al*., 2002; Rwego *et al*., 2008; Causse *et al*., 2015;

Rochelle-Newall *et al*., 2016). Decrease in *E. coli* population due to environmental die-off during application of manure can be estimated using the equation $C = C_i - C_i(R_d\ x\ t)$ where C stands for current *E. coli* concentration in soil sample, C_i stands for initial *E. coli* population in soil sample after application of manure, R_d stands for inactivation rate and t stands for time since application of manure. According to Ribolzi *et al*. (2016), the equation can be applied to evaluate the concentration of *E. coli* in connected receiving water given that the transfer rate between *E. coli* and soil is known or at least 89% of *E. coli* comes from surface runoff. On the contrary, animal population (in agricultural area where rearing livestock are the main activity) can be used to estimate the input of *E. coli*. The equation is $I = C \times \frac{(N_p \times w_i)}{A}$ where I stands for the input of *E. coli* in a specific area over a specific period of time; C is a constant where different size and type of animals are taken into consideration, N_p is the population number of endotherms, W_i is the waste excreted by each individual endotherms, and A is the areas of inhabitation (Rochelle-Newall *et al*., 2016). The equation can only estimate the addition of *E. coli* over a specific period of time but not the total *E. coli* populations, inactivation and die-off rates as discuss before. If the area that animals lived in can be expanded, then the input of *E. coli* population can be decrease remarkedly due to inverse correlation between land use and concentration of *E. coli*. Therefore, the concentration of *E. coli* can be reduced not by reducing the number of animal but expanding the nibbling area. Similarly, the equation can be applied to newly deposited *E. coli* stream water given that the transferral rate of *E. coli* among soil and associated stream water is known or more than 89% of the *E. coli* population comes from runoff (Ribolzi *et al*., 2016). Leaking of waste water infrastructure and increased in runoff due to storm events are two main factors that causes the predication of increase in *E. coli* concentration

complicated (Causse *et al*., 2015; Wu *et al*., 2016; Gotkowska-Plachta *et al*., 2016; Wilson & Weng, 2010). Fewtrell and Kay (2015) found that to quantify the effects of wastewater infrastructure leaks on *E. coli* concentration is challenging because predicting leaks from wastewater structure is difficult. To calculate the contribution of *E. coli* into a region through leaking, we can multiply the fecal concentration of leaks with specific discharge and the removal of bacteria in soil due to filtering effect.

vi) Salinity. Survival of *E. coli* in high osmotic pressure caused by increased in NaCl is challenging. The effect of salinity on *E. coli* includes imbalance osmotic pressure between environment and cytoplasmic, disrupting indigenous bacterial community and more. It was found that *Acidobacteria* and *Deltaproteobacteria* positively correlate with *E. coli*, while *Gammaproteobacteria* and *Flavobacteria* negatively correlate with *E. coli* (Han *et al*., 2021). *Acidobacteria* and *Deltaproteobacteria* are able to utilize low molecular weights of carbon source, thereby encourage carbon turnover rate and this mechanism enable *E. coli* to be able to use carbon source easily (Westphal *et al*., 2011). Meanwhile, *E. coli* shares the same habitat as *Gammoproteobacteria*. Therefore, an increase in *Gammaproteobacteria* population will inhibit the growth of *E. coli*. Westphal *et al*. (2011) also found that *Flavobacteria* exhibit control over growth of *E. coli*. In addition, Han *et al*. (2011) showed that salinity has a positive effect on *Gammaproteobacteria* and *Flavobacteria* while exerting a negative effect on *Acidobacteria* and *Deltaproteobacteria*.

Adaptation of *E. coli* in seawater or changes in osmotic stress has been observed thru uptake or synthesis of organic osmolyte, or control of ion pumps on membranes (Balaji *et al*., 2005). It requires a lot of energy and time to synthesize

organic osmolyte, therefore transporting ions in and out of cells are the preferred option. These organic osmolytes are polar and water soluble in cell. They will not interfere with cellular function even at a high concentration and able to deal with denaturing factors like salt, heat, freezing and thawing and urea (Uysal *et al*., 2022). Example of organic osmolytes include glycine betaine, proline, ectoine, carnitine, choline and trehalose. Previous study done by Killham and Firestone (1984) showed that proline is effective in combating osmotic pressure, while another study was done by Santos and Da Costa (2002) showed that glycine betaine has crucial role in maintaining osmotic equilibrium.

The increase in potassium (K^+) influx is known as the trademark for bacterial response towards changes in osmotic pressure (Balaji *et al*., 2005) due to it being the most abundant inorganic ion in cell. Expression level of *kup/trkD* which mediate K^+ transport across membrane was seen to react to NaCl stress.

Aside from K^+ influx system, stress from starvation also prepares *E. coli* for adverse environments including solar radiation, temperature and osmotic stress. Starvation under stationary phase prepared *E. coli* to be more resistance to stresses than logarithmic phase due to the synthesis of Pex protein during stationary phase.

vii) pH. Microorganisms regularly experienced an array of acid environment in host or nature and they are able to sense and response to acid stress. Acid stress is defined as biological effect of H^+ ion from pH and organic acid in environment from fermentation of food waste or waste in general. Inactivation mechanism of inorganic and organic acid is different for microorganism but both can affect or damange the biochemical process in microorganism. Proton leakage in cell is faster than ability of cell to maintain homeostasis in extreme pH. pH value inside cell increases after organic acid enters cell through cell membrane, the lower the

external pH, the more influx of acid. Constant influx of organic acid will use up cellular energy, thereby causing cell death (Bearson *et al, 1997)*.

According to Gorden and Small (1993), EIEC, ETEC and EHEC were more acid tolerant than commensal strains. According to Lin *et al* (1995), tolerance towards acid can be pH dependent, pH independent or both. Two pH dependent mechanism were induced in *E. coli*, one is in log phase while the other was in stationary phase. Acid habituation is the survival of *E. coli* in acid environment during log phase, which has increased resistance with successive exposure to low pH. Other substance can induce inhabitation during log phase as well, for example glucose, glutamate, phosphate, aspartate, $FeCl_3$ and KCl (Foster, 2000).

Both pathogenic and non-pathogenic *E. coli* were more resistant to acid in stationary phase than log phase. Audia *et al* (2001) identified 3 acid resistance (AR) in stationary phase of *E. coli* and it is dependent on type of medium and growth condition. The acid resistance includes an oxidative system (AR1), two fermentative acid resistance containing glutamate decarboxylate (AR2) and an arginine decarboxylate (AR3).

During growth to stationary phase, AR1 can be induced in Luria Bertani (LB) broth and repressed by glucose. During an event where low pH is present, no amino acid is required for AR1. The oxidative system is dependent on σ^s, while arginine and glutamine acid resistance depend partially on σ^s. The accumulation of proteins in growth medium is associated with glutamate (AR2) and arginine (AR3) decarboxylate mechanism (Rowbury *et al*., 1999). The amino acid decarboxylation plays a part in regulating internal pH by acting as pH homeostasis system. Proton is being utilized during decarboxylation, thereby increasing the pH value. Resistance to acid in pathogenic and non-pathogenic may be stimulate in gastrointestinal tract of cattle. Lin *et al*. (1996) found that at least one of the 3

mechanisms is always active to protect *E. coli* under stationary phase in naturally occurring acidic environment.

Another mechanism to counter acid stress is to change cell membrane composition which is the membrane cyclopropane fatty acid content. Jordan *et al* (1999) reported that a correlation was found between acid resistance and high level of phospholipid containing cyclopropane fatty acid in *E. coli*. Moreover, pathway to repair essential cellular component and homeostasis systems for internal pH has been reported to counter acid stress (DeBiase *et al*, 1999).

viii) Solar radiation. The relationship between solar radiation and survival or inactivation of *E. coli* was predominantly focus on marine waters (Davies-Colley *et al*., 1994; Sinton *et al*., 1999). However, a study by Whitman *et al.* (2004) showed a decreased in *E. coli* concentration during sunny day at Lake Michigan, USA and a decrease in *E. coli* inactivation during cloudy day. Concentration of *E. coli* was found to be more than 235 CFU mL^{-1} during cloudy or partly cloudy day but on sunny day, the concentration was within limit of safe swimming criteria. Similar finding from Maraccini *et al*. (2016) showed that first-order decay rate for *E. coli* was constant for 1 to 2 days with low light conditions and 6 days with high light conditions. Two mechanisms were discussed by Jozić *et al*. (2014), firstly direct solar radiation breaks DNA structures and bond in bacteria cell, and secondly damaged caused by indirect photosensitised reactions by endogenous or exogenous sensitisers.

In addition, depth under water where *E. coli* receive solar radiation also plays a role in the decay rate. A study conducted by Whitman *et al*. (2004) showed that concentration of *E. coli* at 45cm underneath water surface is lower than concentration of *E. coli* at 90cm below water surface. Muela *et al.* (2000) stated

that distinctive component of sunlight can induce different responses in *E. coli*. For instant, high correlation ($\alpha < 0.05$) between first-order decay rate of *E. coli* and ultraviolet B-ray (UVB) was reported. Short exposure (6 hours) to UVB is enough to decrease culturability and activity of *E. coli.* On the contrary, being exposed to ultraviolet A-ray or photosynthetically active radiation (PAR) decreases the culturability of *E. coli* to about 10%. Whitman *et al*. (2004) came out with a formula to calculate the concentration of *E. coli* at 45cm and 90cm beneath water level, the calculations were $\gamma_{45} = 48091e^{-0.4682t}$ and $\gamma_{90} = 12746e^{-0.4184t}$, where γ stands for concentration of *E. coli* (CFU 100mL^{-1}) and t stands for time (hour). Therefore, Whitman *et al*. (2004) suggested that by calculating the exposure time under water is more accurate than isolation.

ix) Suspended solids. The survival of *E. coli* in water could also be affected by suspended solids or sediments and how readily *E. coli* attached to the particles (Grossart, 2010). Particles are able to provide nutrients (organic matter) and protection against radiation by shielding *E. coli* (Drummond *et al*., 2015). In addition to protection against UV radiation, attachment to particles can protect *E. coli* from predator as well. Different microorganisms attached to particle are able to carry out horizontal gene transfer and generation of resistance gene (Corno *et al*., 2014). If more than one microorganism associate in the same particle, the likelihood of horizontal gene transfer is somewhat higher than free floating microorganisms. Microorganisms may acquire resistance to antibiotics, photosynthetically active radiation, ultraviolet lights, predators, etc. (Mamane, 2008; Tang *et al*., 2011; Callieri *et al*., 2016). However, suspended particles, including sediments do not show significant effect on *E. coli* population in second

habitat since most studies also include temperature fluxes which affects *E. coli*'s population more (Czajkowska *et al*., 2005).

Pachepsky and Shelton (2011) came up with an equation to calculate the survivability of *E. coli* in sediments, the equation is $\ln C = lnC_0 - \mu_t$ where C is the current concentration, C_0 is initial concentration, μ represents inactivation rate and t is time. Different inactivation rate has been reported by researchers conducting experiments at different location. Pachepsky and Shelton (2011) reported inactivation rate of 0.54 d^{-1}, Jamieson *et al* (2004) reported at 0.15 d^{-1} and Anderson *et al* (2005) reported at 0.07 d^{-1}. Sediment size also plays a factor in temperature inactivation rate where smaller size is less sensitive to warmer temperature.

x) Predation. Outside of host environment, *E. coli* population will interact with indigenous microbiota in a loose or complex way. Therefore, various types of interactions can be anticipated in different environment (intestinal guts, manure, soil, water or plants). One of the factors is protozoa, which normally known as predation, can be detrimental to *E. coli* but sometimes, will boost survivality too (Van Elsas *et al*., 2011). It is shown by Barker *et al.* (1999) where *E. coli* O157 interact with *Acanthamoeba polyphaga* in the environment. However, most predation, competition for substrate and antagonism on *E. coli* will have a negative effect on *E. coli* population due to cumulative effect (Semenov *et al*., 2007). Without these factors, *E. coli* are able to increase their population in natural habitats.

Van Elsas *et al*. (2007) found that the population dynamic of *E. coli* is being regulated by the diverse indigenous microbial communities. Based on this view, Trevor (1998) found that ecosystems with more diverse biodiversity is more

resistant towards disturbance than lower biodiversity. Infiltration by *E. coli* population into the former habitats will be challenging than latter habitat due to resistance towards disturbance. Even though the exact impact of autochthonous microbial community on survival of *E. coli* population is still unclear, but this aspect is considered to be important. According to Van Elsas *et al*. (2007), the survival of foreign *E. coli* population in inversely proportional to the diversity of microbial community present in soil. Changes in the diversity of microbial population and community composition was seen to have positive effect on the increased survival rate of introduced *E. coli* (Van Elsas *et al*., 2011).

The importance of protozoan in soil, water and sewage is their role in controlling the microorganism's population. Protozoan are commonly found in water, soils and effluent. They tend to form an important reservoir for spreading of pathogenic strains. These pathogenic strains include *Vibrio, Legionella, Mycobacteria,* enteropathogenic *E. coli* which are found to be able to survived in *Acanthamoeba polyphaga* (Huws *et al*., 2008). Aside from enhancing survivability, co-existing with protozoa could also encourage changes in adaptation to environment (Barker *et al*., 1993).

Chekabab *et al*. (2012) found that persistent of *E. coli* O157: H7 increased to more than 3 weeks when grow together with *A. castellanii*. Furthermore, an increase in mutant that do not produce Stx gene was found to have transient internalization and intracellular survival. When *E. coli* was co-cultured with *A. castellanii*, an increase in expression of virulence genes was observed like Stx, LEE, and non-LEE T3SS effector (Carruthers *et al*., 2010). This imply that pathogenic factors in EHEC may contribute to persistency and survivability when interact with amoeba. Siddiqui and Khan (2012) found that amoeba and human macrophages share similar structural characteristic and function, particularly in phagocytic action and

corresponding mechanism in interaction among microorganism pathogens. Therefore, it was suggested that amoeba could be the essential step in the evolution of environmental bacteria into human pathogens.

However, pathogenic strains which encode Stx killed ciliate protozoa by means of holotoxin Stx as anti-protozoan defence system (Lainhart *et al*., 2009). Studies conducted by Meltz Steinberg and Levin (2007) also demonstrate that the fitness of *E. coli* increases in the presence of Stx-encoding prophage during co-culture with ciliate protozoa. They also found that after 3 days, ratio of Stx+ to Stx- ratio increases when co-culture with *Tetrahymena thermophila* which belongs to protozoa found in ruminants' gut. On the other hand, Burow *et al*. (2005) did not find any changes in fitness of pathogenic *E. coli* when co-culture with rumen protozoa.

The ability of pathogenic *E. coli* to survive and grow in sewage and waste effluents has become a concern, especially when *E. coli* O157:H7 can survive in manure slurry for more than a week (LeJeune *et al*., 2001; Lee *et al*., 2009). Presence of protozoa in effluent could offer a safe ecological niche for pathogens like *E. coli* O157:H7 but the role of protozoa on *E. coli* in natural environment is less studied. According to Rodriguez-Zaragoza (1994), large number of protozoa like *Acanthamoebae* are found in soil contaminated with sewage waste and feces. It is probable that *E. coli* are being preyed on by free-living protozoan and use these protozoans as a vector to spread to other hosts or environment. This scenario is made possible by the survival of bacteria in amoeba cyst form and has been shown by Steinert *et al*. (1998), Brown and Barker (1999) that *Legionella pneumophila, Vibrio cholera,* and *Mycobacterium avium* exercise this method of survival. Aerosol transmission for bacteria is made possible by dispersal of amoeba cysts. Cattle could also be a route of transmission for *E. coli* during grazing activity

whereby protozoan containing pathogenic *E. coli* are being ingested together with grass or feed.

xi) Stress response. Factors above can contribute stress to *E. coli* and these stress responses varied between pathogenic and non-pathogenic *E. coli* (Leenanon and Drake, 2011). Survival under unfavorable or severe condition may happened due to stress response. Enhance in virulence might occur as well. *E. coli* developed signal transduction system to detect changes in environmental stresses and involved gene expression in cellular defense mechanism (Kennelly and Potts,1996). These advances in protective network enable *E. coli* to adapt to environment or survive the unfavorable condition. Sigma factors are the common regulatory mechanism, which binds to RNA polymerase (RNAP). RNAP has 5 subunit (α_1, α_2, β, β', ω) and a sigma factor which factor (σ) which reduces insignificant binding to nonspecific DNA while increase affinity to certain promoter region.

Under non-stress condition, the σ factor, σ^{70} is accountable for transcription of genes promoters while a different σ^{70} with promoter specificity is induced. The master regulator of stress response in *E. coli* and some bacteria is σ^s (RpoS) and level of RpoS is low under non-stress condition. RpoS protein is frequently degraded by ClpXP protease (Schweder *et al, 1996)*. With the regulatory mechanism, the level of σ^s is lower than σ^{70} in non-stress cells.

Response to heat shock is a protective mechanism to handle heat-induced damaged on bacteria which is mediated by σ^{32}, a set of proteins call heat shock proteins (HSPs) are being synthesized. Under stress condition, momentary increase and stabilization of σ^{32} transcription occur, which normal is unstable. Function of HSPs in *E. coli* is to bind to non-native polypeptides and proteins and

prevent aggregation of proteins due to denaturation. HSPs also support protein refolding and assembly (Georgopoulos and Welch, 1993). In addition, some HSPs also assist in digesting denatured proteins that has no function in cell. Besides removal of denatured proteins, HSPs support nucleic acid synthesis, cell divison and motility under normal and stress condition (Morris, 1993). Low level of HSPs are required at low temperature, low level of σ^{32} are found in *E. coli* (Craig and gross, 1991). In a slight increase in temperature (30°C to 42°C), stabilization of σ^{32} occur and synthesis of HSPs increase 10- to 15- fold in 5 minutes then decrease after 30 minutes (Nagai *et al*, 1991). Importance of σ^{32} is demonstrated by Zhou *et al* (1988) where *E. coli* mutant (lacking of σ^{32}) could not grow above 20°C. While σ^{32} plays a role in protecting the host from cytoplasmic damage, σ^{24} protects the cell against extracytoplasmic heat damage or heat stresses. Synthesis of proteins mostly stopped under extreme heat stress due to inactivation of σ^{70} while production of HSPs is regulated by σ^{32} (Blaszczak *et al*., 1995). De Las Pea *et al* (1995) found that genes induced by σ^{24} provide crucial information related to protein folding especially under extreme temperature. Hence, HSPs plays a role in survival of *E. coli* under temperature stresses.

xii) Formation of biofilm. When outside of the hosts, some strains of *E. coli* are able to form biofilm on biotic and abiotic surfaces (Torres *et al*., 2005). Example of abiotic surfaces including glass, stainless steel and polystyrene while biotic surfaces are epithelial cell layer, gallstones and place surfaces (Rivas *et al*., 2007; Franz *et al*., 2007). According to Allison *et al*. (2012), the formation of biofilm involves the production of curli and long polar fimbriae, embed in extracellular polymeric substances (EPS). In addition, biofilm matrix consists of live and dead microorganisms, polysaccharides, lipopolysaccharides (LPS), minerals and DNA.

It is a complex process which involved genetic mechanism, colonic acid and elements encoded by O island. In addition, the formation of biofilm is also associated with expression of pathogenic genes including gene on virulence plasmid pO157 (Puttamreddy *et al*., 2010). Moreover, formation of biofilm is also associated with intercellular signalling molecules, like autoinducer-2 and indole (Yoon & Sofos, 2008). The first step in formation of biofilm in plant stomata or internal tissue involves the attachment of T3SS, flagella and pilus curli on surfaces (Berger *et al*., 2010; Saldana *et al*., 2011). The flagella and pilus curli found on the surface of bacterial cells usually contain adhesins at the end which corresponded to different carbohydrates. Types of fimbriae and adhesins includes type 1, P, S and F1C fimbriae (Yaron and Romling, 2014). According to Wagner and Hensel (2011), the adhesins interact through hydrophobic or electrostatic non-specific binding or specifically bind to mammalian cell receptor, and are accountable for adhesion to certain host. Aside from mammalian cell, curli found on *E. coli* also plays a part in attachment to sprouts and leaves, with low inactivation rate. Deletion of *csg* gene only reduce binding not more than 1-log (Yaron and Romling, 2014). The role of these adhesins on plant cells are not widely studied. Type 1 fimbriae are usually found in *Enterobacteriaceae* which specifically bind to mannosides in glycoproteins on mammalian cells. Furthermore, antigen 43m, a type of adhesins secreted by *E. coli*, binds to the integrins of animal tissue through RGD motifs (Henderson and Owen, 1999).

Aside from the plant stomata, 10^6 to 10^9 per cm^3 of roots can also be found due to rich nutrients that support growth (Hallmann *et al*., 2001). The soil surrounding plant roots contains root mucus as a product of root cell metabolism or cell lysis. These secretion from roots contains organic acid, amino acids, vitamins and polysaccharides which are essential substrates for bacteria growth. Water are

being drawn to mucus and this helps to create a hydrated environment for roots and bacteria. Some bacteria are able to travel to upper parts of the plants by colonizing the roots and invade xylem vessels (Kutter *et al*., 2006; Klerks *et al*., 2007). Compared to plant roots, nutrients are limited on foliage area. Moreover, fluctuation in temperature, solar radiation and water availability creates less concentration of bacteria per gram of leaf (Hallmann *et al*., 2001). According to Beattie and Lindow (1999), foliage bacteria may utilize two main strategies for their survival. A tolerance strategy whereby they develop resistance to environmental stresses or an avoidance strategy whereby they look for suitable habitat to protect them from these stresses. Based on this idea, a model for leaf colonization was developed whereby bacteria adhered to the surface and starts to form microcolonies which continue to form biofilm. Meanwhile, some penetrates into internal spaces and form their own habitat.

Biofilm provides protection for *E. coli* growth outside of host environment by protecting them from bacteriophage and free-living amoeboid predators (Costerton *et al*., 1995). Moreover, cells inside biofilm have better resistant towards chemical and environmental stresses. Various mechanisms contribute to biofilm associated cells increased in resistance, coupled with genetic properties of bacteria itself. For instance, environmental stresses like solar radiation, differential osmotic pressure and desiccation are being protected by presence of EPS. EPS also act as a physical barrier against antimicrobial agents (Del Pozo & Patel, 2007). In various ecosystem, *E. coli* appeared in their planktonic form and will start to form biofilm when nutrients become available. Chekabab *et al.* (2013) state that formation of biofilm for growth in environmental water is predominant as the planktonic population constitute less than 0.1% of the total community. Critical first step in biofilm formation is attachment, the initial attachment is

described as a weak and reversible binding due to hydrophobic or electrostatic interactions. This lasted for few seconds and a firm attachment is established since it is hard to remove attached bacteria (Dunne, 2002; Laus *et al*., 2005). After attachment, biofilm matrix is being form by bacteria which constitute of proteinaceous components and exopolysaccharides (Yaron & Romling, 2014). A thin aggregative fibre which is also known as amyloid fimbriae is a protein component that is encoded by seven or more genes in *csgBAC* and *csgDEFG* operons.

Assembly of curli on cell surface which uses three proteins are being encoded by *csfDEFG*, which also encode for CsgD, a major regulator for transcription that is needed for expression of curli and biofilm formation (Robinson *et al*., 2006). The expression of matrix components and CsgD regulator is regulated by environmental factors like nutrients, oxygen, temperature, osmolarity and regulatory proteins (Gerstel & Romling, 2003). Expression of *csgD* gene is maximum during stationary phase with limited nutrients like nitrogen, phosphate and iron which requires stationary phase RpoS. CsgD expression is also regulated by OmpR, one of the components in OmpR/EnvZ that corresponds to osmolarity (Yaron & Romling, 2014). According to Monteiro *et al*. (2012), RNA chaperone (Hfq) is also a regulator for formation of biofilm in *E. coli*.

The biofilm matrix consists of more than 80 distinct capsules which can be categorized into several groups. O-antigen capsules, which falls under Group 4 capsules, is structurally similar to O-polysaccharides of LPS. They are being polymerized by Wxy polymerase and then transferred across membrane by Wxz complex (Whitfield & Robers, 1999). The over expression of exopolysaccharides (*pgaA* and *aggR* genes) in addition to the regulation of type 1 aggregative

adherence fimbriae could cause *E. coli* O157: H7 to form a stable and thicker biofilm that facilitate the fenugreek seed outbreak in 2011 (Safadi *et al*., 2012).

According to Monier and Lindow (2005), bacteria that is introduced to biotic or abiotic surface, will form aggregates or biofilms. However, they have better survivability when deposited on existing bacteria. For example, *Wausteria paucula* boosted the growth of *E. coli* O157: H7 on lettuce leave (Cooley *et al*., 2006). A study done by Liu *et al*. (2014) showed that *E. coli* O157:H7 encouraged the formation of biofilm from certain strong biofilm-producing species like *Burkholderia caryophylli* and *Ralstonia insidiosa*. The growth of *E. coli* O157: H7 increased by 1 log when incorporate into the dual-species biofilms. This showed that native microorganisms may assist in attachment and survival of foodborne pathogen.

Although there is no concrete link between formation of biofilm and presence or survival of *E. coli*, several studies showed that *E. coli* obtained from coastal sediment seemed to survive. Wang *et al.* (2011) showed that *E. coli* are able to persist and survived in effluent when growing in biofilm. Furthermore, Skandamis *et al*. (2009) also found that *E. coli* in biofilm has increased resistance or tolerance to acid in slaughter plants. In addition, *E. coli* found around farm and livestock area has resistance to fumigation. Fumigation is a process whereby microbial diversity is decrease and this process would favour *E. coli* as there would be no competition for nutrients and space (Ibekwe & Ma, 2011). Therefore, microorganisms in the environment contributes to the survivability of *E. coli* as well.

Biofilm can provide an environment for synthropic interaction between *E. coli* and other microorganisms as well. Whereby *E. coli* can depend on other

microorganisms for utilization of substrate, Uhlich *et al*., 2010 found that non-biofilm forming *E. coli* interact with strains that form biofilm on a solid surface.

CHAPTER 3 : METHODOLOGY

3.1 Study sites

A total of 15 sampling stations were chosen and samples were collected from November 2014 to August 2016 for a total number of 20 samplings. The selected stations were listed in Table 3.1 and labelled in Figure 3.1.

Table 3.1 : Location of the sampling sites in this study. KBU: Kampung Batu Udang; KKG: Kampung Kuala Gris; MU: Manik Urai; KM: Kampung Merbau; KK:Kampung Kuala Krai; TM: Tanah Merah; PM: Pasir Mas; KBH: Kota Bahru; KB: Kuala Besar; KW16: Offshore 1, KW19: Offshore 2; CBC: Cahaya Bulan Beach; MB: Melawi Beach; BC:Bachok Beach; SR: Semarak River.

Station	Sites	Location
KBU	Kg. Batu Udang	5°17′48.4″N, 102°01′10.4″E
KKG	Kg Kuala Gris	5°23′28.0″N, 102°04′5.8″E
MU	Manik Urai	5°23′16.6″N, 102°14′12″E
KM	Kg. Merbau	5°29′29.6″N, 102°11′33″E
KK	Kuala Krai	5°31′53″N, 102°11′47.7″E
TM	Tanah Merah	5°46′38.46″N, 102°09′2.34″E
PM	Pasir Mas	6°01′22.3″N, 102°09′14.1″E
KBH	Kota Bharu	6°07′40.1″N, 102°14′2.2″E
KB	Kuala Besar	6°12′20.7″N, 102°14′3.9″E
KW16	KW 16	6°13′52.83″N, 102°14′19.27″E
KW19	KW 19	6°18′45.42″N, 102°15′43.44″E
CCB	Cahaya Bulan Beach	6°11′53.58″N, 102°16′14.80″E
MB	Melawi Beach	6°01′17.2″N, 102°25′12.0″E
BB	Bachok Beach	6°0′32.97″N, 102°25′35.75″E
SR	Semarak River	5°53′24.3″N, 102°28′49.5″E

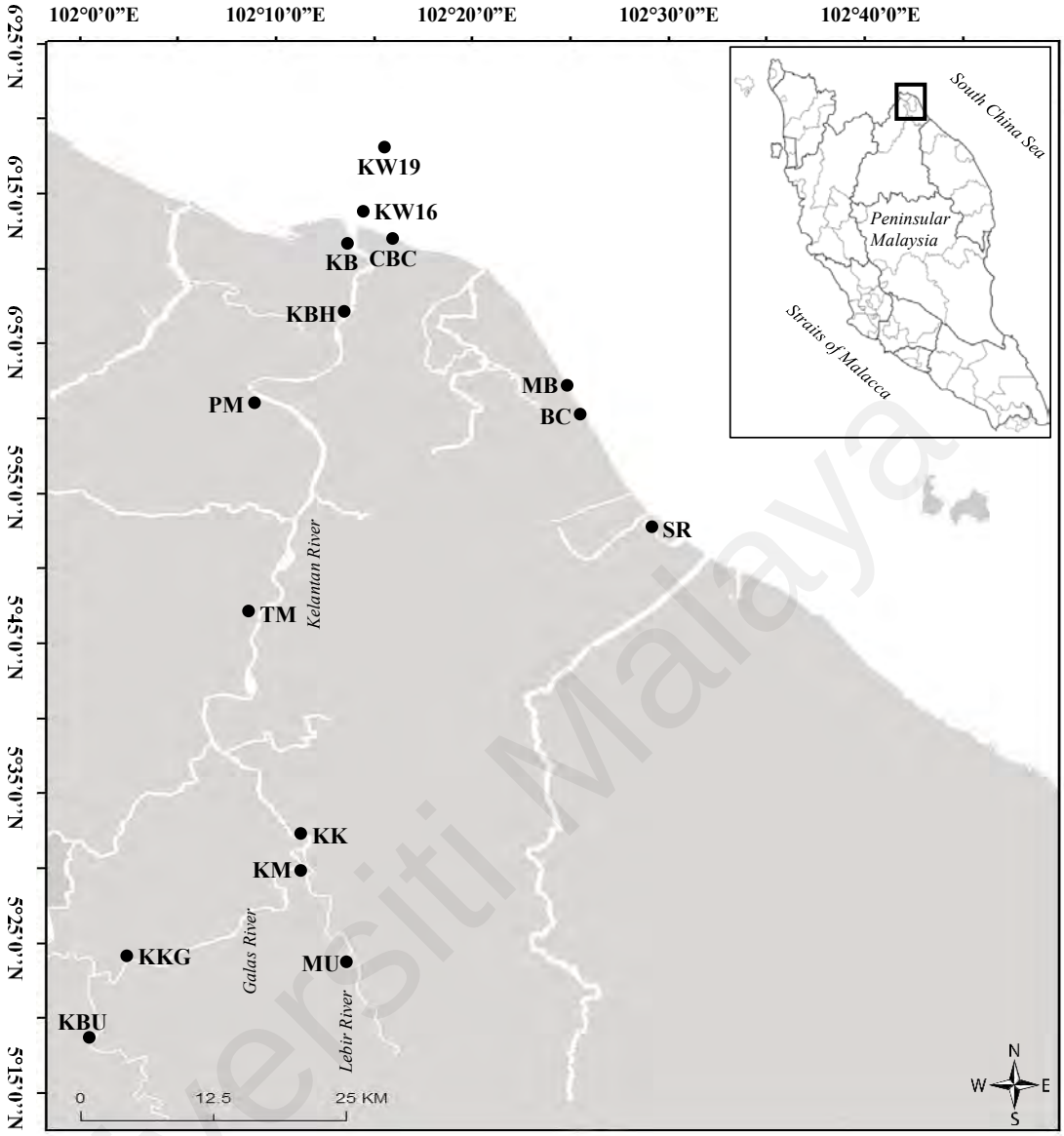

Figure 3.1 : Map of Kelantan River and its adjacent water with sampling sites.

Samples were collected in sterile 2 L Schott bottles in replicates and kept in cold (4°C) while transported back to the laboratory for further analysis. In situ parameters were measured in this study including temperature and salinity using YSI Professional 30 (Yellow Spring, Ohio) while pH was measured using pH meter (Thermo Scientific Orion 4-Star, USA).

3.2 Total Suspended Solids and Particulate Organic Matters

Five hundred mL of water sample was filtered through precombusted GF/F filters (500°C, 3 hours) and filtrate was kept in -20°C for inorganic nutrient analysis. Total suspended solid (TSS) was measured by filtering water sample through a pre-weighted and pre-combusted (500°C for 3 hours) 0.7-µm-pore-size Whatman GF/F filter; these filters were re-weight after drying at 60ºC for 7 days. The same filter was later combusted in furnace (500°C) for 3 hours and weight loss was calculated as particulate organic matter (POM). Formula for TSS and POM were as follow:

$$TSS\ (mg\ L^{-1}) = \frac{Filtered\ dried\ weight\ -\ Prefiltered\ weight}{Volume\ filtered}$$

$$POM\ (\mu g\ L^{-1}) = \frac{Combusted\ weight\ - Prefiltered\ weight}{Volume\ filtered}$$

3.3 Dissolved Oxygen measurement

Dissolved oxygen (DO) concentration was determined using Winkler method (Grasshoff et al., 2009). Water sample was collected in DO bottle (no air bubbles) with known volume (V) and added in Manganese (II) chloride solution and alkaline iodide solution immediately after. DO bottle was sealed and inverted up and down for 20 times to ensure complete mixing and kept still for 2 hours. Thereafter, 3 mL was pipetted from surface layer into a titration flask. Hydrochloric acid (HCl) was added into DO bottle to dissolve the precipitation. After that, content of DO bottle was poured into titration flask and titrated with sodium thiosulphate ($NaSO_4$). The total volume of $NaSO_4$ (T) used was recorded and DO was calculated based on the following formula:

$$Dissolved\ Oxygen\ (\mu M)\ = \frac{0.01\ \times 250\ \times 10^3 \times T}{(V\ - 0.6)} \times f$$

3.4 Dissolved Inorganic Nutrients

Dissolved inorganic nutrients were also being analysed using colorimetric method (Lee *et al*., 2020). Water sample was then filtered through 0.45µm GF/F filter paper and filtrate was stored in -20°C. Analysis of phosphorus concentration was carried out by measuring absorbance at 880 nm. Phosphate form 12-molybdophosphoric acid under acidic condition and then reduced to phosphomolybdenum blue by ascorbic acid together with the presence of potassium antimonyl tartate.

Concentration of ammonium was measured at 640 nm where phenol and hypochlorite reacted with ammonium to form indophenol blue and intensified by sodium nitroprusside. Analysis of silicate was performed by adding acid molybdate reagent for the silicates to form silicomolybdic acid. The presence of phosphate ions in water samples would also react with acid molybdate. Therefore, the addition of ascorbic acid reduced silicomolybdic acid to molybdenum blue complex and absorbance was measured at 810nm. Oxalic acid was added to break down phosphomolybdic complex.

Nitrite reacted with sulfanilamide and α-naphthyl-ethylenediamine hydrochloric acid to form azo dye which had absorbance at 543 nm. For nitrate measurement, nitrate was first reduced by granulated cadmium prior to addition of sulfanilamide and α-naphthyl-ethylenediamine hydrochloric acid. The increase in nitrite concentration was assumed to be the concentration of nitrate.

3.5 Enumeration of *E. coli*

Isolation of *E. coli* was carried out using membrane filtration method, 10mL of water sample was filtered on a sterile 0.45 µm nitrocellulose membrane (Millipore, Ireland). The membrane was then placed on CHROMagar ECC (in triplicates) and incubated at 37°C for 24 hours. Blue colonies formed (presumptive *E. coli*) on CHROMagsar ECC were picked and purified on CHROMagar ECC before store in glycerol forms.

Enumeration of *E. coli* was carried out by counting the number of blue colonies and recorded as CFU $100mL^{-1}$.

3.6 DNA template preparation

Cell boiling method was used to extract crude DNA from pure isolated culture. A single colony of *E. coli* was inoculated to 100 µl of sterile ultrapure water in 1.5ml Eppendorf tube. The tube was then placed on a dry heat bath at 95°C for 10 mins and placed on ice for 10 mins. The tube was then placed on a centrifuge machine and short spin at 5000 rpm for 10 seconds. The crude DNA was used as a template for PCR.

3.7 Identification of *E. coli*

Identification of presumptive *E. coli* was confirmed via detection of *phoA* gene (housekeeping gene) by PCR (Yu and Thong, 2009). Reaction mixture was carried out in 25 µL volume consisting of 1X green buffer (5X green GoTaq reaction buffer, pH 8.5), 0.5 U of *Taq* DNA polymerase (Promega, Madison, Wis, USA), 1 mM of $MgCl_2$, 140 µM of each deoxynucleoside triphosphate (dNTP), 0.1 µM of each primer and 5 µl of DNA. The PCR was performed under the following condition: initial denaturation at 94°C for 2 minutes, 40 cycles of 30 seconds at 92°C, 30 seconds at 59°C and 30 seconds at 72°C; and a final extension for 5 mins at 72°C. Primer sequence previously described by Yu and Thong (2009) was used in this study, they were *PhoA-F* (5'-GTCACAAAAGCCCGGACACCATAAATGCCT-3') and *PhoA-R* (5'-TACACTGTCATTACGTTGCGGATTTGGCGT-3') which produced 903bp fragment.

3.8 Phylogenetic group determination

Triplex PCR was used to assess the phylogenetic groups (A, B1, B2 and D) of *E. coli* (Clermont et al., 2000). Primers pairs used are ChuA.1 (5'-

GACGAACCAACGGTCAGGAT-3') and ChuA.2 (5'-TGCCGCCAGTACCAAAGACA-3'), YjaA.1 (5'-TGAAGTGTCAGGAGACGCTG-3') and YjaA.2 (5'-ATGGAGAATGCGTTCCTCAAC-3') and TspE4C2.1 (5'-GAGTAATGTCGGGGCATTCA-3') and TspE4C2.2 (5'-CGCGCCAACAAAGTATTACG-3') which generated 279, 211 and 152 bp fragment, respectively. Each reaction was performed in 20 µL volume consisting of 1x Green buffer (5X green GoTaq reaction buffer, pH 8.5), 0.5 U of *Taq* DNA polymerase (Promega, Madison, Wis, USA), 120 µM of each deoxynucleoside triphosphate (dNTP), 1.5 mM of $MgCl_2$, 5 µL of DNA, 0.4 µM of *jyaA* primers and 0.24 µM of each *ChuA* and TSPE4-C2 primers. The PCR was performed under the following condition: initial denaturation for 5 minutes at 94°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C; final extension at 72°C for 7 minutes. Characterization of *E. coli* was based on the presence and absence of *ChuA*, *YjaA* and TspE4.C2 as described in Table 3.2.

Table 3.2 : Characterization of *E. coli* phylogroup based on presence or absence of primers.

Phylogenetic group	Primer		
	ChuA	***YjaA***	**TspE4.C2**
A	-	+	-
	-	-	-
B1	-	-	+
	-	+	+
B2	+	+	+
	+	+	-
D	+	-	+
	+	-	-

3.9 Virulence genes detection

Two different multiplex PCR assay (M1 & M2) were carried out for all confirmed *E. coli* to detect virulence genes that were associated with *E. coli* strains causing intestinal infections (Chapman et al., 2006). For both multiplex PCR, reaction mixture was carried out in 25 µL volume consisting of 1x Green buffer (5X green GoTaq reaction buffer, pH 8.5), 0.5 U of *Taq* DNA polymerase (Promega, Madison, Wis, USA), 1.65 mM of $MgCl_2$, 220 µM of each deoxynucleoside triphosphate (dNTP), 0.24 µM of each primer, and 5 µl of DNA. Primer sequence and PCR amplification condition described by Gómez-Duarte et al. (2009) were used. The primer sequences used for virulence genes detection in this study are shown in Table 3.3 and PCR steps as follow: initial denaturation for 2 minutes at 94°C, 40 cycles of 30 seconds at 92°C, 30 seconds at 59°C and 30 seconds at 72°C; and final extension for 5 minutes at 72°C.

Table 3.3 : Primer mix and primer sequence for virulence genes of *E. coli*.

	Primers	Sequence	Expected band
M1	VT.1	5′-GAGCGAAATAATTTATATGTG-3′	518 bp
	VT.2	5′-TGATGATGGCAATTCAGTAT-3′	
	eae.1	5′-CTGAACGGCGATTACGCGAA-3′	917 bp
	eae.2	5′-CGAGACGATACGATCCAG-3′	
	bfpA.1	5′-AATGGTGCTTGCGCTTGCTGC-3′	326 bp
	bfpA.2	5′-GCCGCTTTATCCAACCTGGTA-3′	
	aggR.1	5′-GTATACACAAAAGAAGGAAGC-3′	254 bp
	aggR.2	5′-ACAGAATCGTCAGCATCAGC-3′	
M2	LT.1	5′-GCACACGGAGCTCCTCAGTC-3′	218 bp
	LT.2	5′-TCCTTCATCCTTTCAATGGCTTT-3′	
	ST.1	5′-GCTAAACCAGTAGAG(C) TCTTCAAAA-3′	147 bp
	ST.2	5′-CCCGGTACAG(A) GCAGGATTACAACA-3′	
	daaE.1	5′-GAACGTTGGTTAATGTGGGGTAA-3′	542 bp
	daaE.2	5′-TATTCACCGGTCGGTTATCAGT-3′	
	virF.1	5′-AGCTCAGGCAATGAAACTTTGAC-3′	618 bp
	virF.2	5′-TGGGCTTGATATTCCGATAAGTC-3′	
	ipaH.1	5′-CTCGGCACGTTTTAATAGTCTGG-3′	933 bp
	ipaH.2	5′-GTGGAGAGCTGAAGTTTCTCTGC-3′	

3.10 Statistical Analysis

Paleontological statistics (PAST) software was used to carry out statistical analysis with $p < 0.05$ (95% confidence interval) was accepted as significant (Hammer et al., 2001). Principal component analysis (PCA) was carried out to determine which physicochemical variables affect water quality of Kelantan River and its adjacent coastal water. Canonical correlation analysis (CCA) was also carried out to compare categories of variables measured and phylogenetic groups and the relationship between categories was represented in graphs.

CHAPTER 4 : RESULTS

4.1 Parameters

Table 4.1 shows the physicochemical variables collected for all sampling sites in this study. The surface water temperature for all stations ranged from 29.0 to 31.0°C. Salinity was low in the Kelantan River and gradually increased toward the estuaries (4.4 ± 4.9 – 11.9 ± 11.3) and coastal sites (22.3 ± 8.9 – 30.5 ± 2.1). Meanwhile, average pH value for Kelantan River gradually increased from 7.1 ± 0.5 to 8.2 ± 0.3 (*CV* = 8%) whereas DO was relatively stable across all sites (>270 µM, *CV* = 8%).

In this study, average TSS was strikingly higher in stations along Kelantan River (>150 mg L^{-1}) than estuaries and coastal sites. Similar situation was also observed for POM concentration (>30 mg L^{-1}) along Kelantan River compared to estuaries and coastal sites.

4.2 Dissolved Inorganic Nutrients Analysis

Concentration of dissolved inorganic (ammonium, nitrate, nitrite, silicate and phosphorus) was measured for every site as shown in Table 4.2. Nitrate was the dominant component in total dissolved inorganic nitrogen and its average concentration varied over a wide range from 0.51 µM to 9.80 µM (CV=37%). Higher concentrations were detected in river compared to estuaries and coastal sides with the highest concentration observed at Kuala Krai (9.80 µM). Ammonium concentration was low among sites ranging from 0.65 to 1.65 µM, except for Kota Bahru (10.07 µM). In contrast, nitrate was generally low among all sites (< 0.50 µM). Silicate was detected with a wider range between 14.39 and 263.43 µM. Phosphorus concentration was low, ranging from 0.09 to 0.33 µM.

Table 4.1 : Physiocochemical variables of sampling stations from Kelantan River and its adjacent coastal waters.

River Station	Location	Temperature (˚C)	pH	Salinity (ppt)	DO (μM)	TSS (mg L^{-1})	POM (mg L^{-1})
Kg. Batu Udang (KBU)	5°17′48.4″N, 102°01′10.4″E	29.0±1.7	7.2±0.4	0.4±0.8	320±36	300±241	52±36
Kg. Kuala Gris (KKG)	5°23′28.0″N, 102°04′5.8″E	28.8±2.0	7.2±0.4	0.3±0.6	300±39	259±234	41±31
Manik Urai (MU)	5°23′16.6″N, 102°14′12″E	29.0±1.7	7.2±0.4	0.6±1.1	308±36	233±214	37±22
Kg. Merbau (KM)	5°29′29.6″N, 102°11′33″E	29.3±2.2	7.2±0.4	0.4±0.8	291±17	208±325	32±30
Kuala Krai (KK)	5°31′53″N, 102°11′47.7″E	28.8±2.0	7.1±0.5	0.3±0.4	288±32	286±380	40±36
Tanah Merah (TM)	5°46′38.46″N, 102°09′2.34″E	30.0±2.5	7.3±0.4	0.2±0.4	318±51	236±178	39±21

Table 4.1, continued

River Station	Location	Temperature (˚C)	pH	Salinity (ppt)	DO (μM)	TSS (mg L^{-1})	POM (mg L^{-1})
Pasir Mas (PM)	6°01′22.3″N, 102°09′14.1″E	31.0±2.2	7.3±0.5	0.5±0.8	310±33	249±226	41±28
Kota Bharu (KBH)	6°07′40.1″N, 102°14′2.2″E	30.1±2.0	7.2±0.3	0.7±0.9	309±30	157±102	32±15
Estuaries and Coastal							
Kuala Besar (KB)	6°12′20.7″N, 102°14′3.9″E	30.2±1.7	7.5±0.2	4.4±4.9	309±38	70±37	19±6
KW 16	6°13′52.83″N, 102°14′19.27″E	29.7±1.5	7.8±0.6	11.9±11.3	298±34	92±70	21±10
KW 19	6°18′45.42″N, 102°15′43.44″E	29.6±1.4	8.2±0.3	29.8±3.5	278±24	55±28	17±18

Table 4.1, continued

Estuaries and Coastal	Location	Temperature (˚C)	pH	Salinity (ppt)	DO (µM)	TSS (mg L^{-1})	POM (mg L^{-1})
Cahaya Bulan Beach (CBC)	6°11′53.58″N, 102°16′14.80″E	31.0±1.7	7.8±0.2	28.5±4.2	277±47	101±77	23±14
Melawi Beach (MB)	6°01′17.2″N, 102°25′12.0″E	30.5±2.1	7.9±0.4	30.5±2.1	271±49	135±99	29±22
Bachok Beach (BB)	6°0′32.97″N, 102°25′35.75″E	30.3±1.9	8.1±0.2	30.3±1.9	266±46	121±67	61±143
Semarak (SR)	5°53′24.3″N, 102°28′49.5″E	30.8±2.0	7.7±0.4	22.3±8.9	290±65	88±39	22±9

Table 4.2 : Average nutrient concentration for Kelantan River and its adjacent waters.

Station	Location	Ammonium (µM)	Phosphate (µM)	Silicate (µM)	Nitrite (µM)	Nitrate (µM)
KBU	Kg. Batu Udang	0.65 ± 0.47	0.20 ± 0.09	150.76 ± 82.51	0.18 ± 0.08	8.47 ± 3.85
KKG	Kg. Kuala Gris	1.50 ± 1.50	0.33 ± 0.21	171.30 ± 97.97	0.21 ± 0.11	6.97 ± 1.70
MU	Manik Urai	0.91 ± 0.75	0.19 ± 0.13	152.12 ± 74.27	0.21 ± 0.10	8.80 ± 7.08
KM	Kg Merbau	0.99 ± 1.18	0.19 ± 0.15	263.43 ± 71.71	0.26 ± 0.21	7.52 ± 4.77
KK	Kuala Krai	0.90 ± 0.60	0.28 ± 0.17	156.03 ± 65.91	0.29 ± 0.19	9.80 ± 7.48
TM	Tanah Merah	0.71 ± 0.45	0.29 ± 0.26	143.70 ± 81.50	0.27 ± 0.18	8.48 ± 5.73
PM	Pasir Mas	0.93 ± 0.48	0.24 ± 0.15	214.66 ± 102.77	0.22 ± 0.12	8.08 ± 4.87
KBH	Kota Bahru	10.07 ± 12.38	0.15 ± 0.16	171.30 ± 76.68	0.28 ± 0.16	7.78 ± 3.11
KB	Kuala Besar	1.65 ± 0.87	0.15 ± 0.16	115.26 ± 80.59	0.43 ± 0.54	6.07 ± 2.97
KW16	KW16	1.18 ± 0.81	0.24 ± 0.28	116.35 ± 109.68	0.36 ± 0.43	6.70 ± 3.33
KW19	KW19	0.52 ± 0.45	0.09 ± 0.06	14.39 ± 10.06	0.10 ± 0.11	0.51 ± 0.76
CBC	Cahaya Bulan Beach	1.48 ± 2.33	0.20 ± 0.19	32.18 ± 27.61	0.36 ± 0.34	2.35 ± 1.50

Table 4.2 continued

Station	Location	Ammonium (µM)	Phosphate (µM)	Silicate (µM)	Nitrite (µM)	Nitrate (µM)
MB	Melawi Beach	0.67 ± 0.73	0.11 ± 0.10	11.88 ± 7.44	0.19 ± 0.27	0.80 ± 0.83
BB	Bachok Beach	1.20 ± 1.30	0.08 ± 0.07	11.81 ± 12.21	0.12 ± 0.11	1.73 ± 3.22
SR	Semarak River	4.96 ± 6.04	0.24 ± 0.29	36.52 ± 34.07	0.27 ± 0.21	1.74 ± 1.47

4.3 Enumeration and Isolation of Total Coliform and *E. coli*

The result showed that total coliform counts in the Kelantan River are one to two orders higher than *E. coli* count (Table 4.3 and Figure 4.1). The average coliform concentration ranged from 1.0 x 10^3 to 3.3 x 10^4 CFU 100 mL^{-1} with exception from beaches of Melawi, Bachok and Semarak River which have concentrations of one magnitude higher (2.01 x 10^5, 3.22 x 10^5 and 3.15 x 10^5 respectively). The highest *E. coli* abundance was detected at Kota Bahru site for river site while Semarak River has the highest *E. coli* abundance for estuaries and coastal sites. A total of 2341 *E. coli* had been isolated from the Kelantan River and its adjacent waters.

Table 4.3 : Average concentration of total coliform and *E. coli*.

Station	Location	Total coliform Log (CFU 100mL^{-1})	*E. coli* Log (CFU 100mL^{-1})
KBU	Kg. Batu Udang	4.13 ± 0.36	2.76 ± 0.42
KKG	Kg. Kuala Gris	4.10 ± 0.44	2.74 ± 0.46
MU	Manik Urai	3.97 ± 0.42	2.57 ± 0.55
KM	Kg Merbau	4.06 ± 0.57	2.85 ± 0.69
KK	Kuala Krai	4.11 ± 0.48	2.69 ± 0.50
TM	Tanah Merah	4.03 ± 0.51	2.60 ± 0.47
PM	Pasir Mas	3.92 ± 0.50	2.53 ± 0.44
KBH	Kota Bahru	4.37 ± 0.46	3.45 ± 0.65
KB	Kuala Besar	3.98 ± 0.58	2.80 ± 0.36
KW16	KW16	3.52 ± 0.51	1.80 ± 0.86
KW19	KW19	2.63 ± 0.68	1.18 ± 0.51
CBC	Cahaya Bulan Beach	3.41 ± 0.85	2.40 ± 0.80
MB	Melawi Beach	3.95 ± 1.52	2.60 ± 1.03
BB	Bachok Beach	3.93 ± 1.53	2.87 ± 1.30
SR	Semarak River	4.53 ± 1.06	3.50 ± 1.53

4.4 Identification of *E. coli* and its Phylogenetic Group

A total of 2341 presumptive *E. coli* were picked from the 15 sampling stations. Phylogenetic group B1 dominated with 695 isolates (29.69%), followed by group A with 688 isolates (29.39%) and group D with 597 isolates (25.50%) (Figure 4.2). Phylogenetic group B2 was the least abundant with 361 isolates (15.42%). Phylogenetic groups were not found to be distributed homogeneously (Figure 4.3). Phylogenetic group A was found to be more frequently isolated from river and estuaries compared to adjacent coastal water.

Phylogenetic group A were detected at upstream, Kg. Batu Udang (*n* = 86) followed by Manik Urai (*n*=62). The count decreased until Tanah Merah (*n* = 28) before increasing from the downstream site of Pasir Mas (*n*=70) to the estuaries where the highest recorded (*n* = 129) at Kuala Besar. In contrast phylogenetic group B1 was dominated at the coastal sites with highest abundance recorded at Kg. Merbau (*n* = 91) and the count decreased downstream before increasing at Kota Bharu to the estuary site KW16. Phylogenetic group B2 and D were the least abundant among other groups with highest count observed at Kg. Batu Udang (*n* = 70 and *n* = 103, respectively).

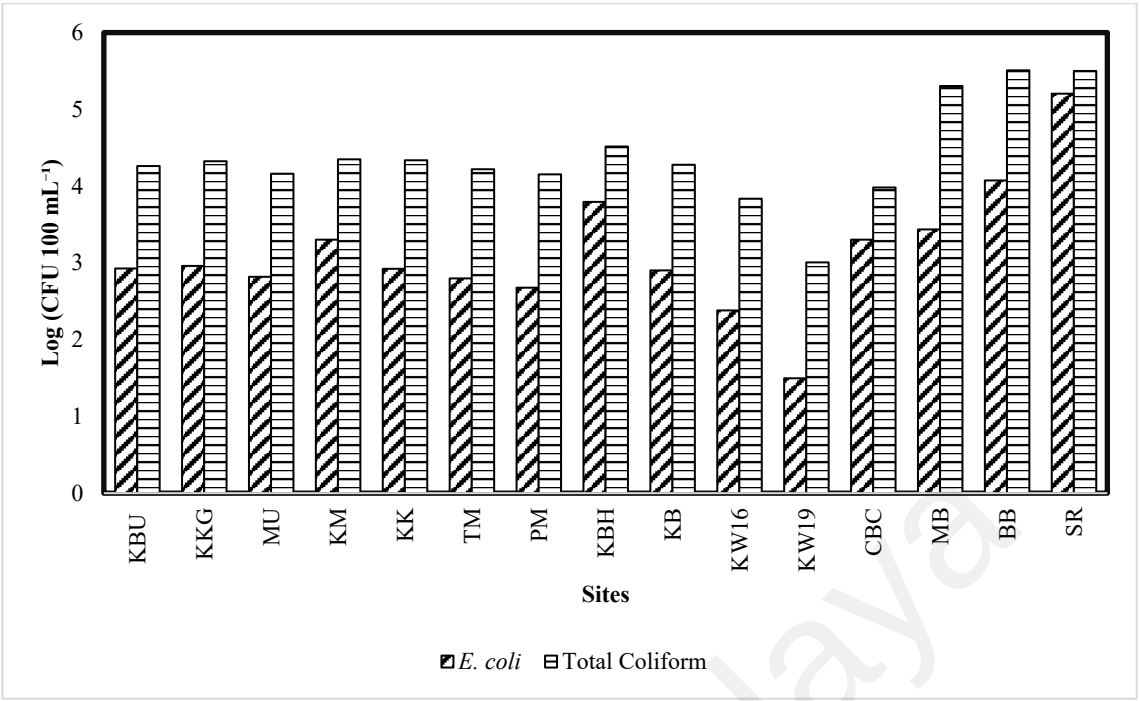

Figure 4.1 : Average Total coliform and *E. coli* Log (CFU 100mL^{-1}) along Kelantan River and its adjacent coastal water.

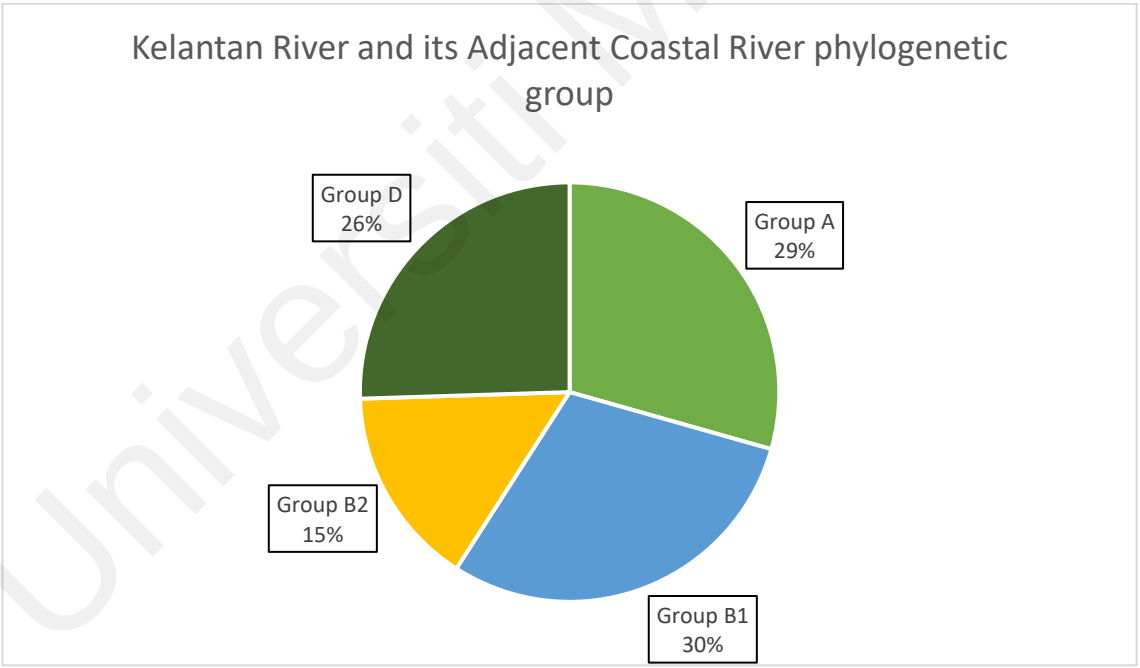

Figure 4.2 : Distribution of each phylogenetic group from Kelantan River and its adjacent coastal water.

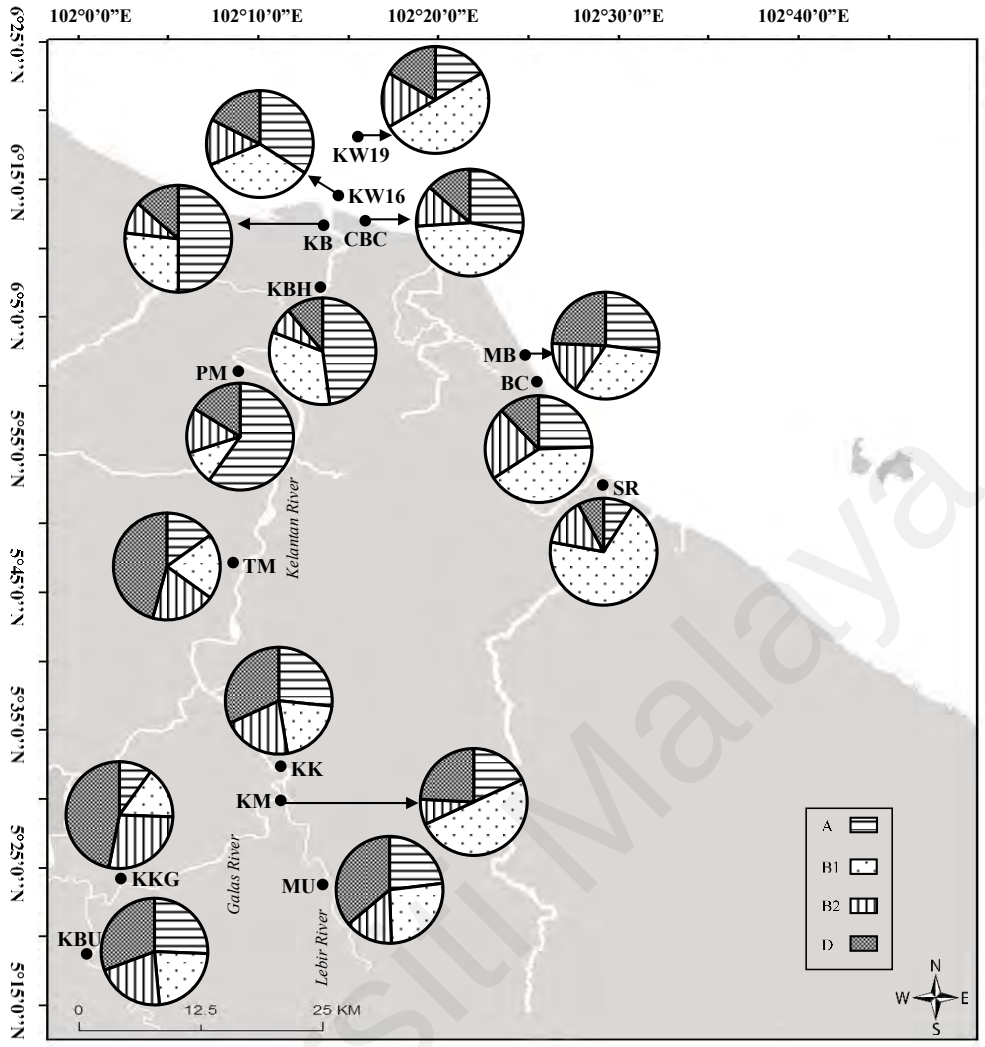

Figure 4.3 : Distribution of phylogenetic group along Kelantan River and its adjacent coastal water.

4.5 Virulence Genes Detection

One isolate from Manik Urai was found to carry *eae* gene while one isolate from Kg. Batu Udang showed the presence of LT gene. Both isolates were not pathogenic strains because they did not possess the required virulent genes for pathogenic strains. For instance, presence of both *eae* and VT genes for EHEC and presence of both LT and ST genes for ETEC.

4.6 Statistical Analysis

Based on the result shown in PCA graph, TSS and SiO_4 were the elements that influence Kelantan River and its adjacent coastal water quality (Figure 4.4). From the graph, PCA 1 and PCA 2 described 72.10% and 19.75% of the total variance of explanatory physical variables measured.

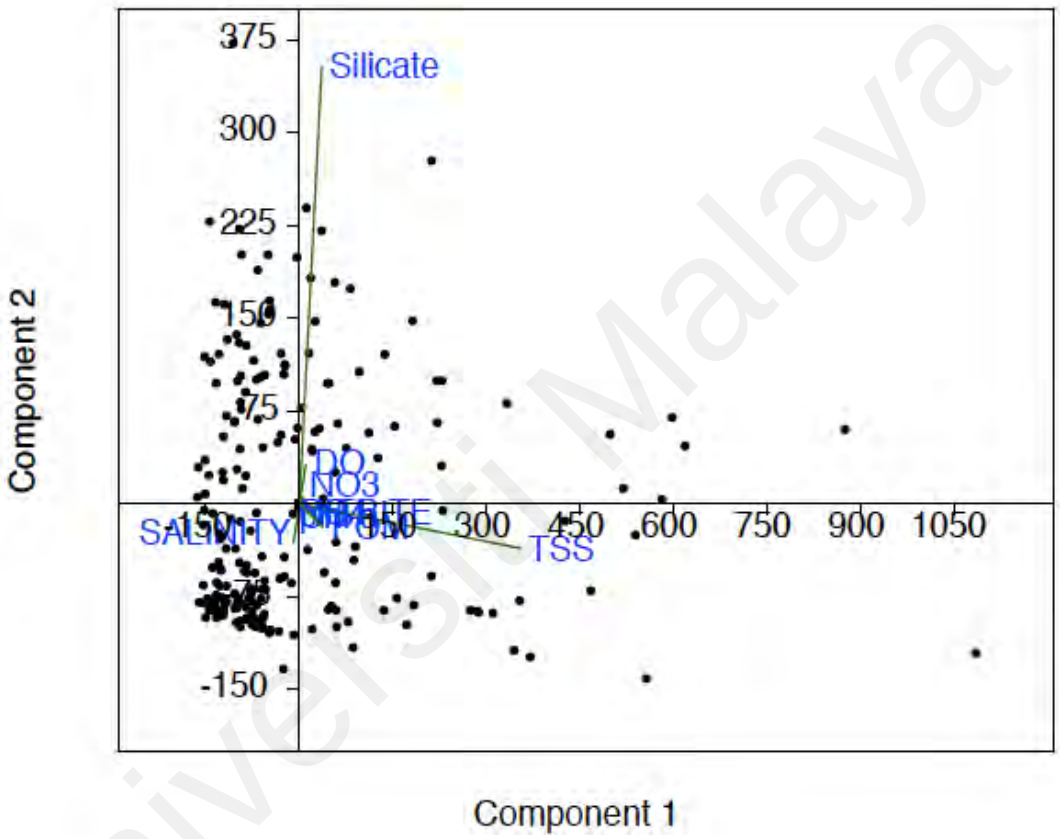

Figure 4.4 : Principal component analysis biplot showing physicochemical variables affecting water quality of Kelantan River and its adjacent coastal water.

CHAPTER 5 : DISCUSSION

5.1 Water Parameters

Surface water temperature recorded in Kelantan River, estuaries and coastal areas were relatively stable among sites. However, salinity varied greatly between river, estuaries and coastal water but were consistent with the studies previously reported for other riverine systems in Malaysia (You et al., 2016; Lim et al., 2018).

High TSS is a prevalent water quality problem in Malaysia (Yen & Rohasliney, 2013). Kelantan River water has been turbid since early 1990s because of logging activities in upstream (Lojing Highlands) (Yen & Rohasliney, 2013). Besides that, deforestation activities, sand mining activities, intensive clearing of land for agriculture and dredging operations have also contributed silt and clay to the river water, causing the river water to turn into a turbid and brownish. The floating fine silt and detritus from the catchment area carried by rainwater during monsoon season (Prasanna & Ranjan, 2010) also increased the TSS and affected the water quality in the Kelantan River Delta. In this study, no correlation was found between TSS and DO (r^2=0.001, $p > 0.10$), suggesting that the TSS in the Kelantan River had generally low organic content (<20%).

Agriculture is the second largest sector of Kelantan in 2016 (Department of Statistic Malaysia, 2017), intensive land use and large-scale planting has increased the use of fertilizer and manure which explained why NO_3 concentration detected in this study was found to be higher than NO_2 [$t(216) = 15.07$, $p < 0.001$] and NH_4 [$t(216)= 8.72$, $p <0.001$]. Similar finding was also reported by Yen & Rohasliney (2013). Samsurijan et al. (2018) has reported that large scale planting of commercial crops (palm oil, rubber, etc.) was seen in Kelantan due to technological development. This caused an increase in the use of fertilizer, manure and soil. Nitrogenous fertilizer accumulates in soils and gradually reaches water bodies through leaching or drainage. Increase in NO_3 could cause algal blooms eutrophication of surface water (Thorburn et al., 2003) and result in health

concerns to animals and humans who rely on groundwater as drinking water (McLay et al., 2001).

Besides agriculture, sewage and small industries effluents also contribute nitrogenous compounds and phosphate in water bodies (Lima et al., 2010). Additionally, anthropogenic activities will introduce higher organic matter and subsequently increase the level of nitrogenous compounds in the river and its adjacent waters (Yen & Rohasliney, 2013; Shamsuddin et al., 2016). In this study, NO_3 concentrations detected was found to be higher than NH_4 indicating less impact of industrial effluents along the Kelantan River, estuaries and its adjacent coasts.

The high SiO_4 concentrations (>13 mg L1) detected in the Kelantan River and the concentrations decreased in the estuaries–coastal waters sites. The main source of SiO_4 is from the mining activities in Kelantan. There are approximately 128 sand mining sites along the Kelantan River and the volume of sand mining increases each year due to high demand (Ambak, 2010). These mining activities begin after the convergence of the two tributaries (Galas River and Lebir River) and continues until the estuary delta of the Kelantan River, which severely impacts the transport and displacement of river sediments (Yen & Rohasliney, 2013).

Moreover, natural and chemical weathering of sedimentary soils and rocks, human activities are also sources of SiO_4 in the river (Shaari et al., 2017; Wang et al., 2017). The decrease in SiO_4 concentration may be attributed to dilution, lack of silica enrichment, utilization of silica by aquatic organisms (e.g. diatoms), and plants that grow along the river (White & Buss, 2014). In this study, the overall inorganic nutrients detected were in range reported in tropical and sub-tropical waters (Lee & Bong, 2006; Sakai et al., 2016).

5.2 Abundance of *E. coli*

Coliform and *E. coli* were highly prevalent in the Kelantan River surface waters, estuaries, and its adjacent coasts. The average abundance of *E. coli* detected at all sampling sites exceeded both the recommended *E. coli* allowable limit by NWQS of class II for rivers in Malaysia (100 CFU/100 ml) and Malaysia IMWQS (Department of Environment, 2019a, 2019b) except KW19. This is indicative of fecal pollution, which is consistent with previous studies on the Kelantan River (Basri et al., 2015; Bamaiyi et al., 2017). Fecal pollution here is mainly due to the direct untreated sewage discharge from the houses and floating toilets built along the riverbanks. According to Department of Statistic (2019), more than half of the wastewater produced by residents in Kelantan did not receive water treatment. The inadequate sewage treatment facilities in Kelantan where the use of individual septic tanks that connect to multipoint of sewage treatment plants (SPAN, 2016) only partially treat the sewage before being discharged into the river (Sakai et al., 2016). The lack of wastewater treatment facilities together with a setup of floating toilets and houses greatly deteriorated the water quality in Kelantan River.

In this study, the average *E. coli* abundance detected in estuarine and coastal sites are one order magnitude higher in contrast to river sites. This may be caused by the untreated sewage released directly from the houses and toilets built near the beach and the coastal upwelling process, which provides a nutrient source and support for *E. coli*. Furthermore, *E. coli* can rapidly adapt to, and tolerate, different abiotic (availability of nutrients, pH, moisture, temperature, salinity) and biotic (grazing) stress factors (Van Elsas et al., 2011; Alves et al., 2014), and could further enhance their fitness in aquatic environments. Studies have shown the capability of *E. coli* to grow and proliferate in marine environments through alkaline pH adaptation (Hughes, 2008) and or change in their genetic structure (Van Elsas et al., 2011). The prevalence of *E. coli* in coastal, estuaries,

and river waters of Kelantan may pose health risks for local residents who have direct or indirect contact with water through recreational activities or seafood consumption.

5.3 Phylogenetic Grouping of *E. coli*

Characterization of *E. coli* in this study into their respective phylogroups revealed that Kelantan River and its adjacent water was dominated by phylogroup B1 and phylogroup A comprised 59% of the total *E. coli* in this study especially at downstream of Kelantan River and coastal sites. These findings are similar to previous studies that reported environmentally persistent *E. coli* are of groups B1 and A rather than virulent types B2 and D (Figueira et al., 2011; Ghaderpour et al., 2015). The ability of phylogroup B1 to form biofilm and higher frequency to utilize sucrose and aromatic compounds provides an advantage to phylogroup B1 survival over other phylogroups in the environment. Similar observation was reported by Perini et al. (2015), whereby phylogenetic group B1 was found to be the most abundant and it was believed that commensal phylogroups are more adapted to the environment. Furthermore, Chakraborty et al. (2015) also found that phylogroup B1 has higher resistance to three or more antibiotic groups. This may be one of the reasons phylogroup B1 can be found in higher abundance compared to other groups.

At upstream sites of the Kelantan River, higher abundances of D phylogroup strains were observed suggesting that fecal contamination was mainly from animal origin (Jakobsen *et al*., 2010). Studies have shown that the *E. coli* population structure differs significantly between humans and animals (Carlos et al., 2010). It has been reported that livestock and poultry are the main reservoir for D phylogroup (Jakobsen *et al*., 2010). Based on statistics from the Department of Veterinary Services Malaysia (2016), poultry farming, with a total of 1.84 million population, is the major livestock activity operating in Kelantan. Poultry could therefore be a major animal fecal pollution source in the river.

The difference between phylogenetic groups among the sites in this study may be attributed to hydrological conditions, different sources of pollution, selective pressures in the waters, and land use (Lyautey et al., 2010; Van Elsas et al., 2011). Distinct survival rates, together with all these parameters, will structure the *E. coli* community distribution and diversity in aquatic environments (Berthe et al., 2013). In this study, a positive correlation was found between *E. coli* and abundance (r^2= 0.165, $p < 0.001$) suggesting that *E. coli* was transported in the river bound to particulate matter. This positive correlation suggested that *E. coli* was transported in the river bound to particulate matter. Research indicates that the attachment of *E. coli* to sediment organic matter with clay content can increase their survival in aquatic environments (Pachepsky & Shelton, 2011; Liang et al., 2017). Suspended solids not only provide organic and inorganic nutrients but also provide protection against adverse factors (ultraviolet radiation, metal toxicity, grazing, attack by bacteriophage) (Medema et al., 2003). On the other hand, secretion of extracellular polymeric substances by microorganisms at the outer cell surface (Liao et al., 2015) for cell aggregation, adhesion, and protection is one of the survival strategies for cells to survive and adapt in hostile environments (Vu et al., 2009; Bruckner et al., 2011).

Besides, TSS was also found to correlate with rainfall (r^2=0.342, $p < 0.001$). This finding concurs with other studies that showed rainfall is the primary process affecting river volume and flow, which can directly increase the level of TSS through runoff (Vaze & Chiew, 2003). This study therefore suggested that rainfall indirectly affects the distribution and abundance of *E. coli* in the Kelantan River, estuaries and its adjacent coasts.

In order to illuminate the factors influencing the *E. coli* phylogroups occurrence and distribution, a CCA was conducted (Figure 5.1). The CCA showed distinct differences in survival among strains belonging to different phylogenetic groups. Phylogenetic group A

was greater in deteriorated water containing NH_4 and NO_2, whereas phylogenetic group D was greatest with NO_3. In contrast, *E. coli* phylogenetic group B2 seemed to thrive in waters with higher DO. Abundance of phylogenetic group B1 appeared to have better salinity tolerance compared to other phylogenetic groups. This explained why phylogenetic group B1 dominated at coastal sites, whereas phylogenetic group D dominated upstream of Kelantan River with higher concentration of NO_3. However, more research is needed to validate these findings.

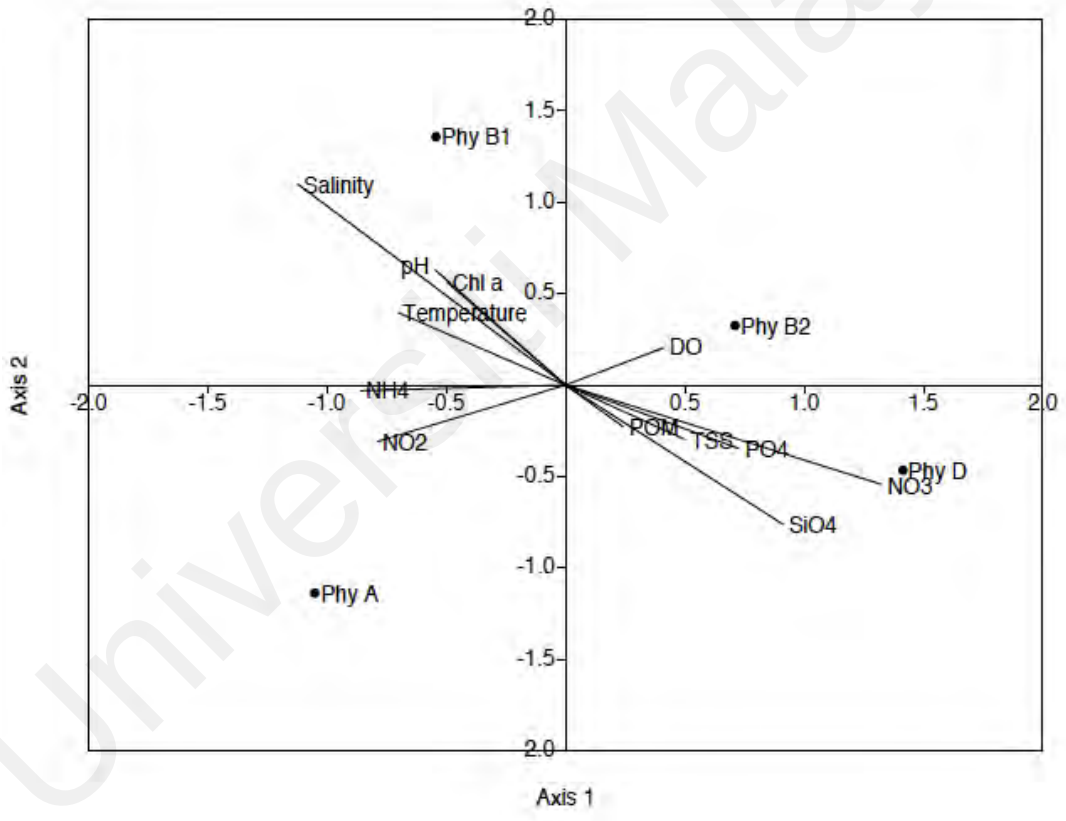

Figure 5.1 : Canonical correlation analysis showing relationship between *E. coli* phylogroups and physicochemical variables.

CHAPTER 6 : CONCLUSION

This study found that NO_3 was the dominant nitrogen species in Kelantan River, estuaries and its adjacent coastal waters. PCA analysis illustrated that TSS and SiO_4 were the physicochemical parameters that influenced the water quality of Kelantan River. Fecal pollution was detected for Kelantan River and its adjacent coastal water due to the counts of coliform and *E. coli* detected exceeded IMWQS (Appendix A) and NWQS (Appendix B) standards. Out of the four phylogroups detected, phylogenetic group A and B1, which were mainly belonged to commensal group, were mostly detected. Whereas phylogenetic group B2 and D were least abundant. TSS was found to be significantly correlated to *E. coli* abundance and rainfall. From CCA analysis, phylogenetic group A and D were able to survive in deteriorating water with NO_2, NO_3 and NH_4 while phylogenetic group B1 seemed to have better tolerance towards salinity. Phylogenetic group B2 appeared to thrive in water with high DO level.

REFERENCES

Ab Razak, N. H., Praveena, S. M., Aris, A. Z., & Hashim, Z. (2016). Quality of Kelantan drinking water and knowledge, attitude and practice among the population of Pasir Mas, Malaysia. *Public Health*, *131*, 103-111.

Adnan, N. A., & Atkinson, P. M. (2011). Exploring the impact of climate and land use changes on streamflow trends in a monsoon catchment. *International journal of climatology*, *31*(6), 815-831.

Ahmad, A., Dada, A. C., Usup, G., & Heng, L. Y. (2013). Validation of the Enterococci indicator for bacteriological quality monitoring of beaches in Malaysia using a multivariate approach. *SpringerPlus*, *2*(1), 1-18.

Allison, S. E., Silphaduang, U., Mascarenhas, M., Konczy, P., Quan, Q., Karmali, M., & Coombes, B. K. (2012). Novel repressor of Escherichia coli O157: H7 motility encoded in the putative fimbrial cluster OI-1. *Journal of bacteriology*, *194*(19), 5343-5352.

Alves, M. S., Pereira, A., Araújo, S. M., Castro, B. B., Correia, A., & Henriques, I. (2014). Seawater is a reservoir of multi-resistant Escherichia coli, including strains hosting plasmid-mediated quinolones resistance and extended-spectrum beta-lactamases genes. *Frontiers in microbiology*, *5*, 426.

Ambak, M. A., & Zakaria, M. Z. (2010). Freshwater fish diversity in Sungai Kelantan. *Journal of Sustainability Science and Management*, *5*(1), 13-20.

Anderson, K. L., Whitlock, J. E., & Harwood, V. J. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Applied and environmental microbiology*, *71*(6), 3041-3048.

Audia, J. P., Webb, C. C., & Foster, J. W. (2001). Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. *International journal of medical microbiology*, *291*(2), 97-106.

Balaji, B., O'Connor, K., Lucas, J. R., Anderson, J. M., & Csonka, L. N. (2005). Timing of induction of osmotically controlled genes in Salmonella enterica serovar Typhimurium, determined with quantitative real-time reverse transcription-PCR. *Applied and environmental microbiology*, *71*(12), 8273-8283.

Bamaiyi, P. H., Izreen, M. S. N., Mohamad, K., Eizzati, B. H. N., Siti-Bainum, C. R., Farhana, M. K. N., ... & Khan, G. K. M. A. (2017). Isolation and polymerase chain

reaction identification of bacteria from the 2014–2015 flood of Kota Bharu, Kelantan, Malaysia. *Asian Biomedicine*, *10*(6), 549-565.

Barker, J., Humphrey, T. J., & Brown, M. W. (1999). Survival of Escherichia coli 0157 in a soil protozoan: implications for disease. *FEMS microbiology letters*, *173*(2), 291-295.

Barker, J., Lambert, P. A., & Brown, M. R. (1993). Influence of intra-amoebic and other growth conditions on the surface properties of Legionella pneumophila. *Infection and Immunity*, *61*(8), 3503-3510.

Basri, Z. D. M., Othman, Z., & Wahid, M. A. (2015). Detection of pathogenic bacteria in flood water. In *ISFRAM 2014* (pp. 185-193). Springer, Singapore.

Bearson, S., Bearson, B., & Foster, J. W. (1997). Acid stress responses in enterobacteria. *FEMS microbiology letters*, *147*(2), 173-180.

Beattie, G. A., & Lindow, S. E. (1999). Bacterial colonization of leaves: a spectrum of strategies. *Phytopathology*, *89*(5), 353-359.

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., & Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental microbiology*, *12*(9), 2385-2397.

Berry, E. D., & Foegeding, P. M. (1997). Cold temperature adaptation and growth of microorganisms. *Journal of Food Protection*, *60*(12), 1583-1594.

Berthe, T., Ratajczak, M., Clermont, O., Denamur, E., & Petit, F. (2013). Evidence for coexistence of distinct *Escherichia coli* populations in various aquatic environments and their survival in estuary water. *Applied and environmental microbiology*, *79*(15), 4684-4693.

Bitton, G. (2005). Microbial indicators of fecal contamination: application to microbial source tracking. *Report submitted to the Florida Stormwater Association*, *719*, 4-6.

Blaszczak, A., Zylicz, M., Georgopoulos, C., & Liberek, K. (1995). Both ambient temperature and the DnaK chaperone machine modulate the heat shock response in Escherichia coli by regulating the switch between sigma 70 and sigma 32 factors assembled with RNA polymerase. *The EMBO Journal*, *14*(20), 5085-5093.

BOBLME (2011). *Country Report on Pollution - Malaysia.* BOBLME 2011-Ecology-11.

Brown, M. R., & Barker, J. (1999). Unexplored reservoirs of pathogenic bacteria: protozoa and biofilms. *Trends in microbiology*, *7*(1), 46-50.

Bruckner, A. W., Faisal, M. O. H. A. M. E. D., & ALNAZRY, H. H. (2011). A paradigm shift for fisheries management to enhance recovery, resilience, and sustainability of coral reef ecosystems in the Red Sea. *Sustainable fisheries: multi-level approaches to a global problem*, 85-111.

Burow, L. C., Gobius, K. S., Vanselow, B. A., & Klieve, A. V. (2005). A lack of predatory interaction between rumen ciliate protozoa and Shiga-toxin producing Escherichia coli. *Letters in applied microbiology*, *40*(2), 117-122.

Byappanahalli, M. N., Whitman, R. L., Shively, D. A., Sadowsky, M. J., & Ishii, S. (2006). Population structure, persistence, and seasonality of autochthonous Escherichia coli in temperate, coastal forest soil from a Great Lakes watershed. *Environmental Microbiology*, *8*(3), 504-513.

Byappanahalli, M., & Fujioka, R. (2004). Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Science and Technology*, *50*(1), 27-32.

Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International journal of environmental research and public health*, *7*(10), 3657-3703.

Callieri, C., Amalfitano, S., Corno, G., & Bertoni, R. (2016). Grazing-induced Synechococcus microcolony formation: experimental insights from two freshwater phylotypes. *FEMS microbiology ecology*, *92*(11).

Camara, M., Jamil, N. R., & Abdullah, A. F. B. (2019). Impact of land uses on water quality in Malaysia: a review. *Ecological Processes*, *8*(1), 1-10.

Carlos, C., Pires, M. M., Stoppe, N. C., Hachich, E. M., Sato, M. I., Gomes, T. A., ... & Ottoboni, L. M. (2010). Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC microbiology*, *10*(1), 1-10.

Carruthers, M. D., Bellaire, B. H., & Minion, F. C. (2010). Exploring the response of Escherichia coli O157: H7 EDL933 within Acanthamoeba castellanii by genome-wide transcriptional profiling. *FEMS microbiology letters*, *312*(1), 15-23.

Causse, J., Billen, G., Garnier, J., Henri-des-Tureaux, T., Olasa, X., Thammahacksa, C., ... & Ribolzi, O. (2015). Field and modelling studies of Escherichia coli loads in tropical streams of montane agro-ecosystems. *Journal of hydro-environment research*, *9*(4), 496-507.

Cennimo, D. J., Koo, H., Mohamed, J. A., Huang, D. B., & Chiang, T. (2007). Enteroaggregative Escherichia coli: a review of trends, diagnosis, and treatment. *Infections in Medicine*, *24*(3), 100.

Chakraborty, A., Saralaya, V., Adhikari, P., Shenoy, S., Baliga, S., & Hegde, A. (2015). Characterization of *Escherichia coli* phylogenetic groups associated with extraintestinal infections in South Indian population. *Annals of medical and health sciences research*, *5*(4), 241-246.

Chandra, M., Cheng, P., Rondeau, G., Porwollik, S., & McClelland, M. (2013). A single step multiplex PCR for identification of six diarrheagenic E. coli pathotypes and Salmonella. *International Journal of Medical Microbiology*, *303*(4), 210-216.

Chapman, T. A., Wu, X. Y., Barchia, I., Bettelheim, K. A., Driesen, S., Trott, D., ... & Chin, J. J. C. (2006). Comparison of virulence gene profiles of Escherichia coli strains isolated from healthy and diarrheic swine. *Applied and environmental microbiology*, *72*(7), 4782-4795.

Chekabab, S. M., Daigle, F., Charette, S. J., Dozois, C. M., & Harel, J. (2012). Survival of enterohemorrhagic E scherichia coli in the presence of A canthamoeba castellanii and its dependence on P ho regulon. *Microbiologyopen*, *1*(4), 427-437.

Chekabab, S. M., Paquin-Veillette, J., Dozois, C. M., & Harel, J. (2013). The ecological habitat and transmission of Escherichia coli O157: H7. *FEMS microbiology letters*, *341*(1), 1-12.

Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and simple determination of the*Escherichia coli* phylogenetic group. *Applied and environmental microbiology*, *66*(10), 4555-4558.

Cooley, M. B., Chao, D., & Mandrell, R. E. (2006). Escherichia coli O157: H7 survival and growth on lettuce is altered by the presence of epiphytic bacteria. *Journal of food protection*, *69*(10), 2329-2335.

Corno, G., Coci, M., Giardina, M., Plechuk, S., Campanile, F., & Stefani, S. (2014). Antibiotics promote aggregation within aquatic bacterial communities. *Frontiers in microbiology*, *5*, 297.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappin-Scott, H. M. (1995). Microbial biofilms. *Annual review of microbiology*, *49*(1), 711-745.

Craig, E. A., & Gross, C. A. (1991). Is hsp70 the cellular thermometer?. *Trends in biochemical sciences*, *16*, 135-140.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic Escherichia coli. *Clinical microbiology reviews*, *26*(4), 822-880.

Csonka, L. N. (1989). Physiological and genetic responses of bacteria to osmotic stress. *Microbiological reviews*, *53*(1), 121-147.

Csonka, L. N., & Epstein, W. (1996). Osmoregulation, p 1210–1223. *Escherichia coli and Salmonella: cellular and molecular biology 2nd ed*, *1*.

Culham, D. E., Lasby, B., Marangoni, A. G., Milner, J. L., Steer, B. A., van Nues, R. W., & Wood, J. M. (1993). Isolation and sequencing of Escherichia coli gene proP reveals unusual structural features of the osmoregulatory proline/betaine transporter, ProP. *Journal of molecular biology*, *229*(1), 268-276.

Czajkowska, D., Witkowska-Gwiazdowska, A., Sikorska, I., Boszczyk-Maleszak, H., & Horoch, M. (2005). Survival of Escherichia coli serotype O157: H7 in water and in bottom-shore sediments. *Polish Journal of Environmental Studies*, *14*(4).

Dasar, K. N., AHMAD, A., Mushrifah, I., & SHUHAIMI-OTHMAN, M. O. H. A. M. A. D. (2009). Water quality and heavy metal concentrations in sediment of Sungai Kelantan, Kelantan, Malaysia: a baseline study. *Sains Malaysiana*, *38*(4), 435-442.

Davies-Colley, R. J., Bell, R. G., & Donnison, A. M. (1994). Sunlight inactivation of enterococci and fecal coliforms in sewage effluent diluted in seawater. *Applied and environmental microbiology*, *60*(6), 2049-2058.

De Biase, D., Tramonti, A., Bossa, F., & Visca, P. (1999). The response to stationary-phase stress conditions in Escherichia coli: role and regulation of the glutamic acid decarboxylase system. *Molecular microbiology*, *32*(6), 1198-1211.

Del Pozo, J. L., & Patel, R. (2007). The challenge of treating biofilm-associated bacterial infections. *Clinical Pharmacology & Therapeutics*, *82*(2), 204-209.

Department of Environment. (2019a). National Water Quality Standards for Malaysia. Retrieved from https://www.doe.gov.my/portalv1/wp-content/uploads/2019/05/Standard-Kualiti-Air-Kebangsaan.pdf

Department of Environment. (2019b). Malaysia Marine Water Quality Standards and Index. Retrieved from https://www.doe.gov.my/portalv1/wp-content/uploads/2019/04/BOOKLET-BI.pdf

Department of Statistic. (2017). GDP by state. Retrieved from https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=102&bul_id=VS9Gckp1UUpKQUFWS1JHUnJZS2xzdz09&menu_id=TE5CRUZCblh4ZTZMODZIbmk2aWRRQT09

Department of Statistic. (2019). Kelantan. Retrieved from https://www.dosm.gov.my/v1/index.php?r=column/cone&menu_id=RU84WGQxYkVPeVpodUZtTkpPdnBmZz09

Department of Veterinary Services Malaysia. (2016). *Bilangan Ternakan 2016* (Number of livestocks 2016). Retrieved from http://www.dvs.gov.my/dvs/resources/user_1/DVS%20pdf/perancangan/2018/Perangkaan%202016%202017/3.Muka_Surat_1-15_.pdf

Desmarais, T. R., Solo-Gabriele, H. M., & Palmer, C. J. (2002). Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied and environmental microbiology*, *68*(3), 1165-1172.

Drummond, J. D., Davies-Colley, R. J., Stott, R., Sukias, J. P., Nagels, J. W., Sharp, A., & Packman, A. I. (2015). Microbial transport, retention, and inactivation in streams: A combined experimental and stochastic modeling approach. *Environmental science & technology*, *49*(13), 7825-7833.

Dunne Jr, W. M. (2002). Bacterial adhesion: seen any good biofilms lately?. *Clinical microbiology reviews*, *15*(2), 155-166.

Ekklesia, E., Shanahan, P., Chua, L. H., & Eikaas, H. S. (2015). Temporal variation of faecal indicator bacteria in tropical urban storm drains. *water research*, *68*, 171-181.

Escobar-Páramo, P., Le Menac'h, A., Le Gall, T., Amorin, C., Gouriou, S., Picard, B., ... & Denamur, E. (2006). Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. *Environmental microbiology*, *8*(11), 1975-1984.

Estrada-Garcia, T., & Navarro-Garcia, F. (2012). Enteroaggregative Escherichia coli pathotype: a genetically heterogeneous emerging foodborne enteropathogen. *FEMS Immunology & Medical Microbiology*, *66*(3), 281-298.

Fagundes-Neto, U., & Scaletsky, I. C. A. (2000). The gut at war: the consequences of enteropathogenic *Escherichia coli* infection as a factor of diarrhea and malnutrition. *Sao Paulo Medical Journal*, *118*(1), 21-29.

Fairbrother, J. M., & Nadeau, E. (2006). Escherichia coli: on-farm contamination of animals. *Rev Sci Tech*, *25*(2), 555-69.

Fauziah, I. A., Hani, M., & Wan Rohaila, W. A. (2014). Status of groundwater contamination in rural area, Kelantan. *Journal of Environmental Science, Toxicology and Food Technology*, *8*(1), 72-80.

Fewtrell, L., & Kay, D. (2015). Recreational water and infection: a review of recent findings. *Current environmental health reports*, *2*(1), 85-94.

Figueira, V., Serra, E., & Manaia, C. M. (2011). Differential patterns of antimicrobial resistance in population subsets of Escherichia coli isolated from waste-and surface waters. *Science of the Total Environment*, *409*(6), 1017-1023.

Fitri, A., Maulud, K. N. A., Rossi, F., Dewantoro, F., Harsanto, P., & Zuhairi, N. Z. (2021, February). Spatial and Temporal Distribution of Dissolved Oxygen and Suspended Sediment in Kelantan River Basin. In *4th International Conference on Sustainable Innovation 2020–Technology, Engineering and Agriculture (ICoSITEA 2020)* (pp. 51-54). Atlantis Press.

Forsythe, S. J., Abbott, S. L., & Pitout, J. (2015). Klebsiella, enterobacter, citrobacter, cronobacter, serratia, plesiomonas, and other enterobacteriaceae. *Manual of clinical microbiology*, 714-737.

Foster, J. W. (2000). Microbial responses to acid stress. *Bacterial stress responses*, 99-115.

Franz, E., Visser, A. A., Van Diepeningen, A. D., Klerks, M. M., Termorshuizen, A. J., & van Bruggen, A. H. (2007). Quantification of contamination of lettuce by GFP-expressing Escherichia coli O157: H7 and Salmonella enterica serovar Typhimurium. *Food Microbiology*, *24*(1), 106-112.

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris, D., & Cormican, M. (2010). Enumeration and characterization of antimicrobial-resistant *Escherichia coli*

bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Applied and environmental microbiology*, *76*(14), 4772-4779.

Garzio-Hadzick, A., Shelton, D. R., Hill, R. L., Pachepsky, Y. A., Guber, A. K., & Rowland, R. (2010). Survival of manure-borne *E. coli*in streambed sediment: effects of temperature and sediment properties. *Water Research*, *44*(9), 2753-2762.

Gasim, M. B., Khalid, N. A., & Muhamad, H. (2015). The influence of tidal activities on water quality of Paka River Terengganu, Malaysia. *Malaysian journal of analytical sciences*, *19*(5), 979-990.

Georgopoulos, C., & Welch, W. J. (1993). Role of the major heat shock protein as molecular chaperones. *Annual review of cell biology*, *9*, 601-634.

Gerstel, U., & Römling, U. (2003). The csgD promoter, a control unit for biofilm formation in Salmonella typhimurium. *Research in microbiology*, *154*(10), 659-667.

Ghaderpour, A., Ho, W. S., Chew, L. L., Bong, C. W., Chong, V. C., Thong, K. L., & Chai, L. C. (2015). Diverse and abundant multi-drug resistant E. coli in Matang mangrove estuaries, Malaysia. *Frontiers in microbiology*, *6*, 977.

Gómez-Duarte, O. G., Bai, J., & Newell, E. (2009). Detection of Escherichia coli, Salmonella spp., Shigella spp., Yersinia enterocolitica, Vibrio cholerae, and Campylobacter spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. *Diagnostic microbiology and infectious disease*, *63*(1), 1-9.

Gorden, J., & Small, P. (1993). Acid resistance in enteric bacteria. *Infection and immunity*, *61*(1), 364-367.

Gordon, D. M., & Cowling, A. (2003). The distribution and genetic structure of Escherichia coli in Australian vertebrates: host and geographic effects. *Microbiology*, *149*(12), 3575-3586.

Gordon, D. M., Stern, S. E., & Collignon, P. J. (2005). Influence of the age and sex of human hosts on the distribution of Escherichia coli ECOR groups and virulence traits. *Microbiology*, *151*(1), 15-23.

Gotkowska-Płachta, A., Gołaś, I., Korzeniewska, E., Koc, J., Rochwerger, A., & Solarski, K. (2016). Evaluation of the distribution of fecal indicator bacteria in a river

system depending on different types of land use in the southern watershed of the Baltic Sea. *Environmental Science and Pollution Research*, *23*(5), 4073-4085.

Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (2009). *Methods of seawater analysis* (3rd ed.). John Wiley & Sons.

Graumann, P. L., & Marahiel, M. A. (1998). A superfamily of proteins that contain the cold-shock domain. *Trends in biochemical sciences*, *23*(8), 286-290.

Griffin, D. M. (1981). Water potential as a selective factor in the microbial ecology of soils. *Water potential relations in soil microbiology*, *9*, 141-151.

Grisaru, S. (2014). Management of hemolytic-uremic syndrome in children. *International journal of nephrology and renovascular disease*, *7*, 231.

Grossart, H. P. (2010). Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed. *Environmental Microbiology Reports*, *2*(6), 706-714.

Hallmann, J., Quadt-Hallmann, A., Miller, W. G., Sikora, R. A., & Lindow, S. E. (2001). Endophytic colonization of plants by the biocontrol agent Rhizobium etli G12 in relation to Meloidogyne incognita infection. *Phytopathology*, *91*(4), 415-422.

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T. A., Fairbrother, J., ... & Brousseau, R. (2007). Occurrence of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from different aquatic ecosystems within the St. Clair River and Detroit River areas. *Applied and environmental microbiology, 73*(2). 477-484.

Hammer, Ø., Harper, D. A., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, *4*(1), 9.

HAMzAH, A. I. N. O. N., Kipli, S. H., Ismail, S. R., Una, R., & Sarmani, S. (2011). Microbiological study in coastal water of Port Dickson, Malaysia. *Sains Malaysiana*, *40*(2), 93-99.

Han, Z., Ma, J., Yang, C. H., & Ibekwe, A. M. (2021). Soil salinity, pH, and indigenous bacterial community interactively influence the survival of E. coli O157: H7 revealed by multivariate statistics. *Environmental Science and Pollution Research*, *28*(5), 5575-5586.

Harrington, S. M., Strauman, M. C., Abe, C. M., & Nataro, J. P. (2005). Aggregative adherence fimbriae contribute to the inflammatory response of epithelial cells

infected with enteroaggregative Escherichia coli. *Cellular microbiology*, *7*(11), 1565-1578.

Hashim, R., Song, T. H., Muslim, N. Z. M., & Yen, T. P. (2014). Determination of heavy metal levels in fishes from the lower reach of the Kelantan River, Kelantan, Malaysia. *Tropical life sciences research*, *25*(2), 21.

Hengge-Aronis, R. (1996). Regulation of gene expression during entry into stationary phase. *Escherichia coli and Salmonella typhimurium*, 1497-1511.

Hooper, L. V., & Gordon, J. I. (2001). Commensal host-bacterial relationships in the gut. *Science*, *292*(5519), 1115-1118.

Hu, J., & Torres, A. G. (2015). Enteropathogenic Escherichia coli: foe or innocent bystander?. *Clinical Microbiology and Infection*, *21*(8), 729-734.

Huang, D. B., Mohanty, A., DuPont, H. L., Okhuysen, P. C., & Chiang, T. (2006). A review of an emerging enteric pathogen: enteroaggregative Escherichia coli. *Journal of medical microbiology*, *55*(10), 1303-1311.

Hughes, B. S. (2008). Evolutionary patterns of *Escherichia coli* growth in seawater determined with a Host to Coast Environmental Laboratory Analog. *Aquatic Microbial Ecology*, *53*(3), 243-255.

Huws, S. A., Morley, R. J., Jones, M. V., Brown, M. R., & Smith, A. W. (2008). Interactions of some common pathogenic bacteria with Acanthamoeba polyphaga. *FEMS microbiology letters*, *282*(2), 258-265.

Ibekwe, A. M., & Ma, J. (2011). Effects of fumigants on microbial diversity and persistence of E. coli O15: H7 in contrasting soil microcosms. *Science of the total environment*, *409*(19), 3740-3748.

Ihssen, J., Grasselli, E., Bassin, C., François, P., Piffaretti, J. C., Koester, W., ... & Egli, T. (2007). Comparative genomic hybridization and physiological characterization of environmental isolates indicate that significant (eco-) physiological properties are highly conserved in the species Escherichia coli. *Microbiology*, *153*(7), 2052-2066.

Ishii, S., & Sadowsky, M. J. (2008). Escherichia coli in the environment: implications for water quality and human health. *Microbes and Environments*, *23*(2), 101-108.

Ishii, S., Ksoll, W. B., Hicks, R. E., & Sadowsky, M. J. (2006). Presence and growth of naturalized Escherichia coli in temperate soils from Lake Superior watersheds. *Applied and environmental microbiology*, *72*(1), 612-621.

Ishii, S., Ksoll, W. B., Hicks, R. E., & Sadowsky, M. J. (2006). Presence and growth of naturalized Escherichia coli in temperate soils from Lake Superior watersheds. *Applied and environmental microbiology*, *72*(1), 612-621.

Ishii, S., Yan, T., Vu, H., Hansen, D. L., Hicks, R. E., & Sadowsky, M. J. (2009). Factors controlling long-term survival and growth of naturalized Escherichia coli populations in temperate field soils. *Microbes and environments*, 0912220144-0912220144.

Isidean, S. D., Riddle, M. S., Savarino, S. J., & Porter, C. K. (2011). A systematic review of ETEC epidemiology focusing on colonization factor and toxin expression. *Vaccine*, *29*(37), 6167-6178.

Jakobsen, L., Kurbasic, A., Skjøt-Rasmussen, L., Ejrnæs, K., Porsbo, L. J., Pedersen, K., ... & Hammerum, A. M. (2010). Escherichia coli isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. *Foodborne pathogens and disease*, *7*(5), 537-547.

Jamieson, R. C., Gordon, R. J., Sharples, K. E., Stratton, G. W., & Madani, A. (2002). Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Canadian biosystems engineering*, *44*(1), 1-9.

Jamieson, R. C., Joy, D. M., Lee, H., Kostaschuk, R., & Gordon, R. J. (2004). Persistence of enteric bacteria in alluvial streams. *Journal of Environmental Engineering and Science*, *3*(3), 203-212.

Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M. N., Yan, T., & Ishii, S. (2017). Environmental Escherichia coli: ecology and public health implications—a review. *Journal of applied microbiology*, *123*(3), 570-581.

Jaureguy, F., Landraud, L., Passet, V., Diancourt, L., Frapy, E., Guigon, G., ... & Picard, B. (2008). Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. *BMC genomics*, *9*(1), 560.

Jiang, W., Hou, Y., & Inouye, M. (1997). CspA, the major cold-shock protein of Escherichia coli, is an RNA chaperone. *Journal of biological chemistry*, *272*(1), 196-202.

Johnson, T. J., & Nolan, L. K. (2009). Pathogenomics of the virulence plasmids of Escherichia coli. *Microbiology and Molecular Biology Reviews*, *73*(4), 750-774.

Jones, D. (1988). Composition and properties of the family Enterobaeteriaceae. *Journal of Applied Bacteriology*, *65*, 1S-19S.

Jordan, K. N., Oxford, L., & O'Byrne, C. P. (1999). Survival of low-pH stress by Escherichia coli O157: H7: correlation between alterations in the cell envelope and increased acid tolerance. *Applied and Environmental Microbiology*, *65*(7), 3048-3055.

Jozić, S., Morović, M., Šolić, M., Krstulović, N., & Ordulj, M. (2014). Effect of solar radiation, temperature and salinity on the survival of two different strains of Escherichia coli. *Fresenius environmental bulletin*, *23*(8), 1852-1859.

Kandror, O., & Goldberg, A. L. (1997). Trigger factor is induced upon cold shock and enhances viability of Escherichia coli at low temperatures. *Proceedings of the National Academy of Sciences*, *94*(10), 4978-4981.

Kang, E., Crouse, A., Chevallier, L., Pontier, S. M., Alzahrani, A., Silué, N., ... & Malo, D. (2018). Enterobacteria and host resistance to infection. *Mammalian genome*, *29*(7), 558-576.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *escherichia coli*. *Nature reviews microbiology*, *2*(2), 123-140.

Kennelly, P. J., & Potts, M. (1996). Fancy meeting you here! A fresh look at" prokaryotic" protein phosphorylation. *Journal of bacteriology*, *178*(16), 4759-4764.

Khairiah, J., Saad, B. S., Habibah, J., Salem, N., Semail, A. B., & Ismail, B. S. (2014). Heavy metal content in soils and vegetables grown in an Inland Valley of Terengganu and a River Delta of Kelantan, Malaysia. *Research Journal of Environmental and Earth Sciences*, *6*(6), 307-312.

Killham, K., & Firestone, M. K. (1984). Salt stress control of intracellular solutes in streptomycetes indigenous to saline soils. *Applied and Environmental Microbiology*, *47*(2), 301-306.

Klerks, M. M., van Gent-Pelzer, M., Franz, E., Zijlstra, C., & Van Bruggen, A. H. C. (2007). Physiological and molecular responses of Lactuca sativa to colonization by Salmonella enterica serovar Dublin. *Applied and environmental microbiology*, *73*(15), 4905-4914.

Knierim, K. J., Hays, P. D., & Bowman, D. (2015). Quantifying the variability in Escherichia coli (E. coli) throughout storm events at a karst spring in northwestern Arkansas, United States. *Environmental earth sciences*, *74*(6), 4607-4623.

Kutter, S., Hartmann, A., & Schmid, M. (2006). Colonization of barley (Hordeum vulgare) with Salmonella enterica and Listeria spp. *FEMS microbiology ecology*, *56*(2), 262-271.

Lainhart, W., Stolfa, G., & Koudelka, G. B. (2009). Shiga toxin as a bacterial defense against a eukaryotic predator, Tetrahymena thermophila. *Journal of bacteriology*, *191*(16), 5116-5122.Mamane, H. (2008). Impact of particles on UV disinfection of water and wastewater effluents: a review. *Reviews in Chemical Engineering*, *24*(2-3), 67-157.

Laus, M. C., Van Brussel, A. A. N., & Kijne, J. W. (2005). Role of cellulose fibrils and exopolysaccharides of Rhizobium leguminosarum in attachment to and infection of Vicia sativa root hairs. *Molecular plant-microbe interactions*, *18*(6), 533-538.

Lee, C. W., & Bong, C. W. (2006). Carbon flux through bacteria in a eutrophic tropical environment: Port Klang waters.*The environment in Asia Pacific harbours* (pp. 329-345). Springer, Dordrecht.

Lee, C. W., Lim, J. H., Heng, P. L., Marican, N. F., Narayanan, K., Sim, E. U. H., & Bong, C. W. (2020). Influence of elevated river flow on hypoxia occurrence, nutrient concentration and microbial dynamics in a tropical estuary. *Environmental monitoring and assessment*, *192*(10), 1-12.

Lee, M. S., Krumpelman, S. L., Apple, J. K., Yancey, J. W. S., Kegley, E. B., Johnson, M. G., ... & Stephens, T. P. (2009). In Vitro and In Vivo Investigations of Antimicrobial Treatments to Reduce Escherichia coli O157: H7 in Cattle Manure. *The Professional Animal Scientist*, *25*(1), 49-59.

Leenanon, B., & Drake, M. A. (2001). Acid stress, starvation, and cold stress affect poststress behavior of Escherichia coli O157: H7 and nonpathogenic Escherichia coli. *Journal of food protection*, *64*(7), 970-974.

LeJeune, J. T., Besser, T. E., & Hancock, D. D. (2001). Cattle water troughs as reservoirs of Escherichia coli O157. *Applied and Environmental Microbiology*, *67*(7), 3053-3057.

Liang, X., Liao, C., Soupir, M. L., Jarboe, L. R., Thompson, M. L., & Dixon, P. M. (2017). Escherichia coli attachment to model particulates: the effects of bacterial cell characteristics and particulate properties. *PloS one*, *12*(9), e0184664.

Liao, C., Liang, X., Soupir, M. L., & Jarboe, L. R. (2015). Cellular, particle and environmental parameters influencing attachment in surface waters: a review. *Journal of applied microbiology*, *119*(2), 315-330.

Liévin-Le Moal, V., & Servin, A. L. (2013). Pathogenesis of human enterovirulent bacteria: lessons from cultured, fully differentiated human colon cancer cell lines. *Microbiology and Molecular Biology Reviews*, *77*(3), 380-439.

Lim, J. H., Lee, C. W., Bong, C. W., Affendi, Y. A., Hii, Y. S., & Kudo, I. (2018). Distributions of particulate and dissolved phosphorus in aquatic habitats of Peninsular Malaysia. *Marine pollution bulletin*, *128*, 415-427.

Lima, M. C., Souza, M. F., Eca, G. F., & Silva, M. A. (2010). Export and retention of dissolved inorganic nutrients in the Cachoeira River, Ilhéus, Bahia, Brazil. *Journal of Limnology*, *69*(1), 138-145.

Lin, J., Lee, I. S., Frey, J., Slonczewski, J. L., & Foster, J. W. (1995). Comparative analysis of extreme acid survival in Salmonella typhimurium, Shigella flexneri, and Escherichia coli. *Journal of bacteriology*, *177*(14), 4097-4104.

Lin, J., Smith, M. P., Chapin, K. C., Baik, H. S., Bennett, G. N., & Foster, J. W. (1996). Mechanisms of acid resistance in enterohemorrhagic Escherichia coli. *Applied and Environmental Microbiology*, *62*(9), 3094-3100.

Luna, G. M., Vignaroli, C., Rinaldi, C., Pusceddu, A., Nicoletti, L., Gabellini, M., ... & Biavasco, F. (2010). Extraintestinal Escherichia coli carrying virulence genes in coastal marine sediments. *Applied and environmental microbiology*, *76*(17), 5659-5668.

Lyautey, E., Lu, Z., Lapen, D. R., Wilkes, G., Scott, A., Berkers, T., ... & Topp, E. (2010). Distribution and diversity of *Escherichia coli* populations in the South Nation River drainage basin, eastern Ontario, Canada. *Applied and Environmental Microbiology*, *76*(5), 1486-1496.

Madic, J., Vingadassalon, N., de Garam, C. P., Marault, M., Scheutz, F., Brugère, H., ... & Auvray, F. (2011). Detection of Shiga toxin-producing Escherichia coli serotypes O26: H11, O103: H2, O111: H8, O145: H28, and O157: H7 in raw-milk cheeses by using multiplex real-time PCR. *Applied and environmental microbiology*, *77*(6), 2035-2041.

Maraccini, P. A., Mattioli, M. C. M., Sassoubre, L. M., Cao, Y., Griffith, J. F., Ervin, J. S., ... & Boehm, A. B. (2016). Solar inactivation of enterococci and Escherichia

coli in natural waters: effects of water absorbance and depth. *Environmental Science & Technology*, *50*(10), 5068-5076.

Marteyn, B., Gazi, A., & Sansonetti, P. (2012). Shigella: a model of virulence regulation in vivo. *Gut microbes*, *3*(2), 104-120.

McLay, C. D. A., Dragten, R., Sparling, G., & Selvarajah, N. (2001). Predicting groundwater nitrate concentrations in a region of mixed agricultural land use: a comparison of three approaches. *Environmental Pollution*, *115*(2), 191-204.

McWilliams, B. D., & Torres, A. G. (2015). Enterohemorrhagic Escherichia coli adhesins. *Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli*, 131-155.

Medema, G. J., Shaw, S., Waite, M., Snozzi, M., Morreau, A., & Grabow, W. (2003). Catchment characterisation and source water quality. *Assessing Microbial Safety of Drinking Water*, *4*, 111-158.

Meltz Steinberg, K., & Levin, B. R. (2007). Grazing protozoa and the evolution of the Escherichia coli O157: H7 Shiga toxin-encoding prophage. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1621), 1921-1929.

Meng, J., LeJeune, J. T., Zhao, T., & Doyle, M. P. (2012). Enterohemorrhagic Escherichia coli. *Food microbiology: Fundamentals and frontiers*, 287-309.

Milliman, J. D., & Farnsworth, K. L. (2013). *River discharge to the coastal ocean: a global synthesis*. Cambridge University Press.

Milne, D. P., Curran, J. C., Findlay, J. S., Crowther, J. M., Bennett, J., & Wood, B. J. B. (1991). The effect of dissolved nutrients and inorganic suspended solids on the survival of E. coli in seawater. *Water Science and Technology*, *24*(2), 133-136.

Mirhoseini, A., Amani, J., & Nazarian, S. (2018). Review on pathogenicity mechanism of enterotoxigenic Escherichia coli and vaccines against it. *Microbial pathogenesis*, *117*, 162-169.

Mishra, M., Arukha, A. P., Patel, A. K., Behera, N., Mohanta, T. K., & Yadav, D. (2018). Multi-drug resistant coliform: water sanitary standards and health hazards. *Frontiers in pharmacology*, *9*, 311.

Monier, J. M., & Lindow, S. E. (2005). Aggregates of resident bacteria facilitate survival of immigrant bacteria on leaf surfaces. *Microbial Ecology*, *49*(3), 343-352.

Monteiro, C., Papenfort, K., Hentrich, K., Ahmad, I., Le Guyon, S., Reimann, R., ... & Römling, U. (2012). Hfq and Hfq-dependent small RNAs are major contributors to multicellular development in Salmonella enterica serovar Typhimurium. *RNA biology*, *9*(4), 489-502.

Morris, J. G. (1993). Bacterial shock responses. *Endeavour*, *17*(1), 2-6.

Muela, A., Garcia-Bringas, J. M., Arana, I., & Barcina, I. (2000). The effect of simulated solar radiation on Escherichia coli: the relative roles of UV-B, UV-A, and photosynthetically active radiation. *Microbial Ecology*, *39*(1), 65-71.

Mushtaq, S., Irfan, S., Sarma, J. B., Doumith, M., Pike, R., Pitout, J., Livermore, D. M., & Woodford, N. (2011). Phylogenetic diversity of Escherichia coli strains producing NDM-type carbapenemases. *Journal of antimicrobial chemotherapy*, *66*(9), 2002-2005.

Na, S. H., Miyanaga, K., Unno, H., & Tanji, Y. (2006). The survival response of Escherichia coli K12 in a natural environment. *Applied microbiology and biotechnology*, *72*(2), 386-392.

Nagai, H., Yuzawa, H., & Yura, T. (1991). Regulation of the heat shock response in E coli: involvement of positive and negative cis-acting elements in translational control of σ32 synthesis. *Biochimie*, *73*(12), 1473-1479.

Nataro, J. P. (2005). Enteroaggregative Escherichia coli pathogenesis. *Current opinion in gastroenterology*, *21*(1), 4-8.

Nazarian, S., Gargari, S. L. M., Rasooli, I., Amani, J., Bagheri, S., & Alerasool, M. (2012). An in silico chimeric multi subunit vaccine targeting virulence factors of enterotoxigenic Escherichia coli (ETEC) with its bacterial inbuilt adjuvant. *Journal of microbiological methods*, *90*(1), 36-45.

Ngah, M. S. Y. C., Hashim, M., Nayan, N., Said, Z. M., & Ibrahim, M. H. (2012). Marine pollution trend analysis of tourism beach in Peninsular Malaysia. *World Applied Sciences Journal*, *17*(10), 1238-1245.

Nowrouzian, F. L., Wold, A. E., & Adlerberth, I. (2005). Escherichia coli strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. *The Journal of infectious diseases*, *191*(7), 1078-1083.

Ochoa, T. J., & Contreras, C. A. (2011). Enteropathogenic E. coli (EPEC) infection in children. *Current opinion in infectious diseases*, *24*(5), 478.

Osman, S., Aziz, N. A., Husaif, N., Sidek, L. M., Shakirah, A., Hanum, F., & Basri, H. (2018). Application of Stochastic Flood Forecasting Model Using Regression Method for Kelantan Catchment. In *MATEC Web of Conferences* (Vol. 203, p. 07001). EDP Sciences.

O'sullivan, G. C., Kelly, P., O'Halloran, S., Collins, C., Collins, J. K., Dunne, C., & Shanahan, F. (2005). Probiotics: an emerging therapy. *Current pharmaceutical design*, *11*(1), 3-10.

Pachepsky, Y. A., & Shelton, D. R. (2011). Escherichia coli and fecal coliforms in freshwater and estuarine sediments. *Critical reviews in environmental science and technology*, *41*(12), 1067-1110.

Pandey, P. K., & Soupir, M. L. (2013). Assessing the impacts of E. coli laden streambed sediment on E. coli loads over a range of flows and sediment characteristics. *JAWRA Journal of the American Water Resources Association*, *49*(6), 1261-1269.

Perini, L., Quero, G. M., García, E. S., & Luna, G. M. (2015). Distribution of Escherichia coli in a coastal lagoon (Venice, Italy): temporal patterns, genetic diversity and the role of tidal forcing. *Water research*, *87*, 155-165.

Petersen, F., & Hubbart, J. A. (2020a). Quantifying Escherichia coli and Suspended Particulate Matter concentrations in a mixed-Land USE Appalachian watershed. *Water*, *12*(2), 532.

Petersen, F., & Hubbart, J. A. (2020b). Physical factors impacting the survival and occurrence of Escherichia coli in secondary habitats. *Water*, *12*(6), 1796.

Petersen, F., Hubbart, J. A., Kellner, E., & Kutta, E. (2018). Land-use-mediated Escherichia coli concentrations in a contemporary Appalachian watershed. *Environmental earth sciences*, *77*(22), 1-13.

Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J., & Denamur, E. (1999). The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. *Infection and immunity*, *67*(2), 546-553.

Pichereau, V., Hartke, A., & Auffray, Y. (2000). Starvation and osmotic stress induced multiresistances: influence of extracellular compounds. *International journal of food microbiology*, *55*(1-3), 19-25.

Pooveneswary, N., Bin Abu Bakar, A. F., Wei, B. C., Weng Lee, C., Ai Jun, W., Amir Hassan, M. H. B., ... & Pauzi Zakaria, M. (2020). Contamination status and ecological risk of heavy metals in surface sediment of Kelantan River and its nearshore area, Malaysia. *Water Supply*, *20*(1), 103-117.

Pradhan, B., & Youssef, A. M. (2011). A 100-year maximum flood susceptibility mapping using integrated hydrological and hydrodynamic models: Kelantan River Corridor, Malaysia. *Journal of Flood Risk Management*, *4*(3), 189-202.

Prasanna, M. B., & Ranjan, P. C. (2010). Physico chemical properties of water collected from Dhamra estuary. *International journal of environmental sciences*, *1*(3), 334-42.

Puttamreddy, S., Cornick, N. A., & Minion, F. C. (2010). Genome-wide transposon mutagenesis reveals a role for pO157 genes in biofilm development in Escherichia coli O157: H7 EDL933. *Infection and immunity*, *78*(6), 2377-2384.

Radzir, N. A. M., Ali, C. A., & Mohamed, K. R. (2016). Bathymetric mapping of shallow coastal area of Sungai Kelantan Delta, Malaysia onwards South China Sea shelf. *SAINS MALAYSIANA*, *45*(12), 1843-1848.

Rahimi, E., KhamesipouR, F., Yazdi, F., & Momtaz, H. (2012). Isolation and characterization of enterohaemorragic Escherichia coli O157: H7 and EHEC O157: NM from raw bovine, camel, water buffalo, caprine and ovine milk in Iran. *Kafkas Univ Vet Fak Derg*, *18*(4), 559-564.

Rathi, A., Thong, K. L., & Chong, V. C. (2009). Isolation, detection and genomic differentiation of *Escherichia coli* from aquatic environments in Kelantan, Malaysia. *Malaysian Journal of Science*, *29*(Sp.), 1-10.

Razzaq, S. (2006). Hemolytic uremic syndrome: an emerging health risk. *American family physician*, *74*(6), 991-996.

Reeve, C. A., Amy, P. S., & Matin, A. (1984). Role of protein synthesis in the survival of carbon-starved Escherichia coli K-12. *Journal of Bacteriology*, *160*(3), 1041-1046.

Ribolzi, O., Cuny, J., Sengsoulichanh, P., Mousquès, C., Soulileuth, B., Pierret, A., ... & Sengtaheuanghoung, O. (2011). Land use and water quality along a Mekong tributary in Northern Lao PDR. *Environmental management*, *47*(2), 291-302.

Ribolzi, O., Evrard, O., Huon, S., Rochelle-Newall, E., Henri-des-Tureaux, T., Silvera, N., ... & Sengtaheuanghoung, O. (2016). Use of fallout radionuclides (7Be, 210Pb) to estimate resuspension of Escherichia coli from streambed sediments during floods in a tropical montane catchment. *Environmental Science and Pollution Research*, *23*(4), 3427-3435.

Ribolzi, O., Evrard, O., Huon, S., Rochelle-Newall, E., Henri-Des-Tureaux, T., Silvera, N., ... & Sengtaheuanghoung, O. (2016). Use of fallout radionuclides (7Be, 210Pb) to estimate resuspension of Escherichia coli from streambed sediments during floods in a tropical montane catchment. *Environmental Science and Pollution Research*, *23*(4), 3427-3435.

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Hebert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T., Blake, P. A., & Cohen, M. L. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine*, *308*(12), 681-685.

Rivas, L., Dykes, G. A., & Fegan, N. (2007). A comparative study of biofilm formation by Shiga toxigenic Escherichia coli using epifluorescence microscopy on stainless steel and a microtitre plate method. *Journal of Microbiological Methods*, *69*(1), 44-51.

Robinson, L. S., Ashman, E. M., Hultgren, S. J., & Chapman, M. R. (2006). Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Molecular microbiology*, *59*(3), 870-881.

Robinson, L. S., Ashman, E. M., Hultgren, S. J., & Chapman, M. R. (2006). Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Molecular microbiology*, *59*(3), 870-881.

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Coenen, S., Cohen, J., Findlay,D., Gyssens, I., Heure, O. E., Kahlmeter, G., Kruse, H., Laxminarayan, R., Liebana, E., Lopez-Cerero, L., MacGowan, A., Martins, M., Rodriguez-Bano, J., Rolain, J. M., … Vila, J. (2015). The global threat of antimicrobial resistance: science for intervention. *New microbes and new infections*, *6*, 22-29.

Rochelle-Newall, E. J., Ribolzi, O., Viguier, M., Thammahacksa, C., Silvera, N., Latsachack, K., ... & Pierret, A. (2016). Effect of land use and hydrological processes on Escherichia coli concentrations in streams of tropical, humid headwater catchments. *Scientific reports*, *6*(1), 1-12.

Rochelle-Newall, E., Nguyen, T. M. H., Le, T. P. Q., Sengtaheuanghoung, O., & Ribolzi, O. (2015). A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. *Frontiers in microbiology*, *6*, 308.

Rodríguez-Zaragoza, S. (1994). Ecology of free-living amoebae. *Critical reviews in microbiology*, *20*(3), 225-241.

Rowbury, R. J., Humphrey, T. J., & Goodson, M. (1999). Properties of an L-glutamate-induced acid tolerance response which involves the functioning of extracellular induction components. *Journal of Applied Microbiology*, *86*(2), 325-330.
Rowbury, R. J., Humphrey, T. J., & Goodson, M. (1999). Properties of an L-glutamate-induced acid tolerance response which involves the functioning of extracellular induction components. *Journal of Applied Microbiology*, *86*(2), 325-330.

Russo, T. A., & Johnson, J. R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and infection*, *5*(5), 449-456.

Rwego, I. B., Gillespie, T. R., Isabirye-Basuta, G., & Goldberg, T. L. (2008). High rates of Escherichia coli transmission between livestock and humans in rural Uganda. *Journal of Clinical Microbiology*, *46*(10), 3187-3191.

Saadatkhah, N., Tehrani, M. H., Mansor, S., Khuzaimah, Z., Kassim, A., & Saadatkhah, R. (2016). Impact assessment of land cover changes on the runoff changes on the extreme flood events in the Kelantan River basin. *Arabian Journal of Geosciences*, *9*(17), 1-17.

Safadi, R. A., Abu-Ali, G. S., Sloup, R. E., Rudrik, J. T., Waters, C. M., Eaton, K. A., & Manning, S. D. (2012). Correlation between in vivo biofilm formation and virulence gene expression in Escherichia coli O104: H4. *PloS one*, *7*(7), e41628.

Sakai, N., Sakai, M., Haron, D. E. M., Yoneda, M., & Mohd, M. A. (2016). Beta-agonist residues in cattle, chicken and swine livers at the wet market and the environmental impacts of wastewater from livestock farms in Selangor State, Malaysia. *Chemosphere*, *165*, 183-190.

Saldaña, Z., Sánchez, E., Xicohtencatl-Cortes, J., Puente, J. L., & Girón, J. A. (2011). Surface structures involved in plant stomata and leaf colonization by Shiga-toxigenic Escherichia coli O157: H7. *Frontiers in microbiology*, *2*, 119.

Samsurijan, M. S., Abd Rahman, N. N., Ishak, M. I. S., Masron, T. A., & Kadir, O. (2018). Land use change in Kelantan: Review of the Environmental Impact Assessment (EIA) reports. *Geografia-Malaysian Journal of Society and Space*, *14*(4).

Santos, H., & Da Costa, M. S. (2002). Compatible solutes of organisms that live in hot saline environments. *Environmental Microbiology*, *4*(9), 501-509.

Satyanarayana, B., Mohamad, K. A., Idris, I. F., Husain, M. L., & Dahdouh-Guebas, F. (2011). Assessment of mangrove vegetation based on remote sensing and ground-truth measurements at Tumpat, Kelantan Delta, East Coast of Peninsular Malaysia. *International Journal of Remote Sensing*, *32*(6), 1635-1650.

Schubert, S., Dufke, S., Sorsa, J., & Heesemann, J. (2004). A novel integrative and conjugative element (ICE) of Escherichia coli: the putative progenitor of the Yersinia high-pathogenicity island. *Molecular microbiology*, *51*(3), 837-848.

Schultz, J. E., Latter, G. I., & Matin, A. (1988). Differential regulation by cyclic AMP of starvation protein synthesis in Escherichia coli. *Journal of bacteriology*, *170*(9), 3903-3909.

Schwaber, M. J., & Carmeli, Y. (2008). Carbapenem-resistant Enterobacteriaceae: a potential threat. *Jama*, *300*(24), 2911-2913.

Schweder, T., Lee, K. H., Lomovskaya, O., & Matin, A. (1996). Regulation of Escherichia coli starvation sigma factor (sigma s) by ClpXP protease. *Journal of bacteriology*, *178*(2), 470-476.

Semenov, A. V., Van Bruggen, A. H., Van Overbeek, L., Termorshuizen, A. J., & Semenov, A. M. (2007). Influence of temperature fluctuations on Escherichia coli O157: H7 and Salmonella enterica serovar Typhimurium in cow manure. *FEMS microbiology ecology*, *60*(3), 419-428.

Servin, A. L. (2005). Pathogenesis of Afa/Dr diffusely adhering Escherichia coli. *Clinical microbiology reviews*, *18*(2), 264-292.

Shaari, H., Shazili, N. A. M., Abdullah, L. I., Abdullah, N. A., Husain, M. L., & Tahir, N. M. (2017). Geochemistry and clay minerals of surface sediments of Southwestern Johor, Malaysia. *Malaysian Journal of Analytical Sciences*, *21*(2), 312-322.

Shamsuddin, A. S., Ismail, S. N. S., Abidin, E. Z., Bin, H. Y., & Juahir, H. (2016). Contamination of nitrate in groundwater and evaluation of health risk in Bachok,

Kelantan: A cross-sectional study. *American Journal of Applied Sciences*, *13*(1), 80.

Siddiqui, R., & Khan, N. A. (2012). Acanthamoeba is an evolutionary ancestor of macrophages: a myth or reality?. *Experimental parasitology*, *130*(2), 95-97.

Siegele, D. A., & Kolter, R. O. B. E. R. T. O. (1992). Life after log. *Journal of bacteriology*, *174*(2), 345-348.

Sinton, L. W., Finlay, R. K., & Lynch, P. A. (1999). Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Applied and environmental microbiology*, *65*(8), 3605-3613.

Skandamis, P. N., Stopforth, J. D., Ashton, L. V., Geornaras, I., Kendall, P. A., & Sofos, J. N. (2009). Escherichia coli O157: H7 survival, biofilm formation and acid tolerance under simulated slaughter plant moist and dry conditions. *Food Microbiology*, *26*(1), 112-119.

Solo-Gabriele, H. M., Wolfert, M. A., Desmarais, T. R., & Palmer, C. J. (2000). Sources of Escherichia coli in a coastal subtropical environment. *Applied and Environmental Microbiology*, *66*(1), 230-237.

Sousa, C. P. (2006). The versatile strategies of *Escherichia coli* pathotypes: a mini review. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, *12*(3), 363-373.

SPAN. (2016). *Water and sewerage*. National Environmental Health Action Plan (NEHAP) Conference, Putrajaya. Retrieved from http://nehapmalaysia.moh.gov.my/wp-content/uploads/2016/03/Paper-1-Water-Sewerage.pdf

Steinert, M., Birkness, K., White, E., Fields, B., & Quinn, F. (1998). Mycobacterium avium bacilli grow saprozoically in coculture with Acanthamoeba polyphaga and survive within cyst walls. *Applied and environmental microbiology*, *64*(6), 2256-2261.

Tang, K. W., Dziallas, C., & Grossart, H. P. (2011). Zooplankton and aggregates as refuge for aquatic bacteria: protection from UV, heat and ozone stresses used for water treatment. *Environmental microbiology*, *13*(2), 378-390.

Tate III, R. L. (1978). Cultural and environmental factors affecting the longevity of Escherichia coli in histosols. *Applied and Environmental Microbiology*, *35*(5), 925-929.

Thomazini, C. M., Samegima, D. A., Rodrigues, M. A., Victoria, C. R., & Rodrigues, J. (2011). High prevalence of aggregative adherent Escherichia coli strains in the mucosa-associated microbiota of patients with inflammatory bowel diseases. *International Journal of Medical Microbiology*, *301*(6), 475-479.

Thorburn, P. J., Biggs, J. S., Weier, K. L., & Keating, B. A. (2003). Nitrate in groundwaters of intensive agricultural areas in coastal Northeastern Australia. *Agriculture, ecosystems & environment*, *94*(1), 49-58.

Torres, A. G., Jeter, C., Langley, W., & Matthysse, A. G. (2005). Differential binding of Escherichia coli O157: H7 to alfalfa, human epithelial cells, and plastic is mediated by a variety of surface structures. *Applied and environmental microbiology*, *71*(12), 8008-8015.

Trabulsi, L. R., Keller, R., & Gomes, T. A. T. (2002). 10.321/eid0805. Typical and Atypical Enteropathogenic *Escherichia coli*. *Emerging infectious diseases*, *8*(5), 508.

Uhlich, G. A., Rogers, D. P., & Mosier, D. A. (2010). Escherichia coli serotype O157: H7 retention on solid surfaces and peroxide resistance is enhanced by dual-strain biofilm formation. *Foodborne Pathogens and Disease*, *7*(8), 935-943.

Uysal, A., Yildiztugay, E., Gunes, E., & Aladag, M. O. (2022). COMBATING OF ESCHERICHIA COLI STRAINS AGAINST SALINITY BY THE ANTIOXIDANT DEFENSE SYSTEM AND OSMOREGULATION: BIOCHEMICAL AND PHYSIOLOGICAL APPROACHES. *Journal of microbiology, biotechnology and food sciences*, e5163-e5163.

Van Elsas, J. D., Semenov, A. V., Costa, R., & Trevors, J. T. (2011). Survival of Escherichia coli in the environment: fundamental and public health aspects. *The ISME journal*, *5*(2), 173-183.

Van Elsas, J. D., Semenov, A. V., Costa, R., & Trevors, J. T. (2011). Survival of Escherichia coli in the environment: fundamental and public health aspects. *The ISME journal*, *5*(2), 173-183.

Vaze, J., & Chiew, F. H. (2003). Comparative evaluation of urban storm water quality models. *Water Resources Research*, *39*(10).

Vu, B., Chen, M., Crawford, R. J., & Ivanova, E. P. (2009). Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*, *14*(7), 2535-2554.

Wagner, C., & Hensel, M. (2011). Adhesive mechanisms of Salmonella enterica. *Bacterial adhesion*, 17-34.

Walk, S. T., Alm, E. W., Calhoun, L. M., Mladonicky, J. M., & Whittam, T. S. (2007). Genetic diversity and population structure of Escherichia coli isolated from freshwater beaches. *Environmental microbiology*, *9*(9), 2274-2288.

Wang, A. J., Bong, C. W., Xu, Y. H., Hassan, M. H. A., Ye, X., Bakar, A. F. A., ... & Loh, K. H. (2017). Assessment of heavy metal pollution in surficial sediments from a tropical river-estuary-shelf system: A case study of Kelantan River, Malaysia. *Marine pollution bulletin*, *125*(1-2), 492-500.

Wang, A., Lin, B., Sleep, B. E., & Liss, S. N. (2011). The impact of biofilm growth on transport of Escherichia coli O157: H7 in sand. *Groundwater*, *49*(1), 20-31.

Weiskel, P. K., Howes, B. L., & Heufelder, G. R. (1996). Coliform contamination of a coastal embayment: sources and transport pathways. *Environmental science & technology*, *30*(6), 1872-1881.

Westphal, A., Williams, M. L., Baysal-Gurel, F., LeJeune, J. T., & McSpadden Gardener, B. B. (2011). General suppression of Escherichia coli O157: H7 in sand-based dairy livestock bedding. *Applied and environmental microbiology*, *77*(6), 2113-2121.

White, A. F., & Buss, H. L. (2014). 7.4-Natural weathering rates of silicate minerals. *Treatise on Geochemistry, Second Edition. Elsevier, Oxford*, 115-155.

Whitfield, C., & Roberts, I. S. (1999). Structure, assembly and regulation of expression of capsules in Escherichia coli. *Molecular microbiology*, *31*(5), 1307-1319.

Whitman, R. L., Nevers, M. B., Korinek, G. C., & Byappanahalli, M. N. (2004). Solar and temporal effects on Escherichia coli concentration at a Lake Michigan swimming beach. *Applied and environmental microbiology*, *70*(7), 4276-4285.

Wilkinson, J., Kay, D., Wyer, M., & Jenkins, A. (2006). Processes driving the episodic flux of faecal indicator organisms in streams impacting on recreational and shellfish harvesting waters. *Water Research*, *40*(1), 153-161.

Williams, A. P., Avery, L. M., Killham, K., & Jones, D. L. (2005). Persistence of Escherichia coli O157 on farm surfaces under different environmental conditions. *Journal of Applied Microbiology*, *98*(5), 1075-1083.

Wilson, C., & Weng, Q. (2010). Assessing surface water quality and its relation with urban land cover changes in the Lake Calumet Area, Greater Chicago. *Environmental Management*, *45*(5), 1096-1111.

Wu, J., Yunus, M., Islam, M. S., & Emch, M. (2016). Influence of climate extremes and land use on fecal contamination of shallow tubewells in Bangladesh. *Environmental science & technology*, *50*(5), 2669-2676.

Wu, S. Y., & Klein, D. A. (1976). Starvation effects on Escherichia coli and aquatic bacterial responses to nutrient addition and secondary warming stresses. *Applied and Environmental Microbiology*, *31*(2), 216-220.

Wu, X., Altman, R., Eiteman, M. A., & Altman, E. (2014). Adaptation of *Escherichia coli* to elevated sodium concentrations increases cation tolerance and enables greater lactic acid production. *Appl. Environ. Microbiol.*, *80*(9), 2880-2888.

Yaron, S., & Römling, U. (2014). Biofilm formation by enteric pathogens and its role in plant colonization and persistence. *Microbial biotechnology*, *7*(6), 496-516.

Yen, T. P., & Rohasliney, H. (2013). Status of water quality subject to sand mining in the Kelantan River, Kelantan. *Tropical life sciences research*, *24*(1), 19.

Yoon, Y., & Sofos, J. N. (2008). Autoinducer-2 activity of gram-negative foodborne pathogenic bacteria and its influence on biofilm formation. *Journal of food science*, *73*(3), M140-M147.

You, K. G., Bong, C. W., & Lee, C. W. (2016). Antibiotic resistance and plasmid profiling of Vibrio spp. in tropical waters of Peninsular Malaysia. *Environmental monitoring and assessment*, *188*(3), 171.

Yu, K. X., & Thong, K. L. (2009). Multiplex PCR for simultaneous detection of virulence genes in *Escherichia coli*. *Malaysian Journal of Science*, *28*(1).

Yucel, N., & Ulusoy, H. (2006). A Turkey survey of hygiene indicator bacteria and Yersinia enterocolitica in raw milk and cheese samples. *Food Control*, *17*(5), 383-388.

Zain, M. M., & Naing, N. N. (2002). Sociodemographic characteristics of food handlers and their knowledge, attitude and practice towards food sanitation: a preliminary report. *Southeast Asian journal of tropical medicine and public health*, *33*(2), 410-417.

Zhang, Z., Deng, Z., & Rusch, K. A. (2012). Development of predictive models for determining enterococci levels at Gulf Coast beaches. *Water research*, *46*(2), 465-474.

Zhou, Y. N., Kusukawa, N., Erickson, J. W., Gross, C. A., & Yura, T. (1988). Isolation and characterization of Escherichia coli mutants that lack the heat shock sigma factor sigma 32. *Journal of Bacteriology*, *170*(8), 3640-3649.