MICROBIOLOGICAL QUALITY OF RAW AND SMOKED *Corbicula fluminea* FROM SELECTED DISTRICTS IN KELANTAN

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by

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Dissertation Submitted in Partial Fulfilment
of the Requirement for the Degree of
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CERTIFICATE

This is to certify that the dissertation entitled "Microbiological Quality of Raw and Smoked *Corbicula fluminea* from Selected Districts in Kelantan" is the bona fide record of research work done by Mohd Sharazi Bin Shahad during the period from March 2020 to September 2020 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted I partial fulfilment for the degree of Master of Science (Forensic Science).

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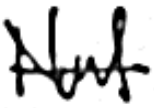

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously for concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, ABBREVIATION & ACRONYMS

-	To/ unit
%	Percentage
/	Over to
=	Equal to
>	More than
≥	More than or equal to
<	Less than
°C	Degree Celsius
CFU	Colony Forming Units
C. fluminea	*Corbicula Fluminea*
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
FCC	Faecal Coliform Count
FDA	Food and Drug Administration
MKKM	*Makmal Keselamatan dan Kualiti Makanan*
MOH	Ministry of Health
RTE	Ready-To-Eat
TCC	Total Coliform Count
TPC	Total Plate Count

KUALITI MIKROBIOLOGI ETAK SALAI DAN MENTAH *Corbicula fluminea* DARI DAERAH TERPILIH DI KELANTAN

ABSTRAK

Corbicula Fluminea atau dikenali sebagai ‘etak’ dalam kalangan warga tempatan di Kelantan, adalah sejenis kerangan air tawar yang boleh ditemui di dasar atau tertanam dalam lumpur di sungai yang cetek. Sebagai penapis air semula jadi, spesis lokan ini terdedah kepada pengumpulan bahan organik merbahaya, besi berat, dan mikroorganisma yang berada disekelilingnya ekosistemnya. Kajian ini dijalankan untuk menentukan kualiti mikrobiologi didalam sampel etak salai dan mentah yang diperolehi dari beberapa penjual di tiga daerah di Kelantan bermula dari Jun sehingga Julai 2020. Secara keseluruhan, 30 sampel telah dianalisis bagi pengiraan plat keseluruhan, pengiraan koliform keseluruhan dan pengiraan koliform najis dan pengesanan kehadiran bakteria *E. coli* didalam sampel tersebut. Kesemua sampel etak mentah (100%) didapati tidak memuaskan untuk analisis pengiraan plat keseluruhan, pengiraan koliform keseluruhan, pengiraan koliform najis dan 73.3% daripada sampel adalah positif dengan kehadiran bakteria *E. coli.* Daripada itu, 60% etak salai didapati tidak memuaskan untuk pengiraan plat keseluruhan, pengiraan koliform keseluruhan, pengiraan koliform najis dan 46.7% menunjukkan kehadiran bakteria *E. coli*. Secara kesimpulannya, etak salai dan mentah yang dijual di daerah Kota Bharu, Pasir Mas dan Bachok didapati tidak memuaskan dan tidak selamat untuk dimakan, dan jaminan keselamatan masih diragui.

MICROBIOLOGICAL QUALITY OF RAW AND SMOKED *Corbicula fluminea* FROM SEVERAL DISTRICTS IN KELANTAN

ABSTRACT

Corbicula fluminea (Asian Clam) or locally known as 'etak' among Kelantanese, is an edible freshwater bivalve found on the surface or buried in sand and mud in the shallow water of riverbeds. As a filter feeder's, bivalves can cause bioaccumulation of hazardous organic matters, heavy metals, and microbes from the surrounding aquatic environment. This study aims to identify the current status for microbiological quality of raw and smoked *Corbicula fluminea* collected from several vendors in three districts in Kelantan from Jun to July 2020. A total of thirty samples were analysed for total plate count (TPC), total coliform count (TCC), faecal coliform count (FCC) and the presence of *E. coli*. All samples of raw *Corbicula fluminea* (100%) samples were found unsatisfactory for TPC, TCC, and FCC and 73.3% of the samples were positive for the presence of *E. coli*. About 60% of smoked *Corbicula fluminea* (n=15) were found unsatisfactory for TPC, TCC and FCC, and 46.7% showed the presence of *E. coli*. In conclusion, the microbiological quality of raw and smoked *C. fluminea* sold in Kota Bharu, Pasir Mas and Bachok is unsatisfactory and unsafe for consumption, and its safety remains doubtful.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Aquaculture is a fast-growing industry in Malaysia due to the high demand for fresh and saltwater products all over the world. Products such as bivalve, river catfish, tilapia and freshwater prawn have significant demand, and they are marketed locally as well as exported. Since 2017, the total production of aquaculture products in Malaysia has increased, and bivalve molluscs have steadily contributed substantially to the market volume (FOA, 2020). The bivalve *Corbicula fluminea* (Asian Clam) or 'etak' (Figure 1.1), as known locally, is a type of edible freshwater bivalve found on the surface or buried in sand and mud in the shallow water of riverbeds. It is indigenous in Australia, South Africa, and South-East Asia countries.

C. fluminea feeds on phytoplankton, green algae, diatoms, zooplankton, and protozoan as its primary food sources (Sousa *et al*., 2008; Mohamad Shamsul *et al*., 2017; Dee *et al*., 2019;; Yusof *et al*., 2020). As a filter feeder's, clams can cause bioaccumulation of hazardous products such as organic matters, heavy metals, and microbes (Khan Chowdhury *et al*., 2009; Sow *et al.*, 2019). It can filter up to one litre of water per hour individually through its gills. Various types of microorganisms are commonly found in the gastrointestinal tract of bivalves. It is greatly influenced by those microorganisms found from the surrounding environment, especially when wastewater or sludge were discharged directly into the river (Sicuro, 2015; Ngiam *et al*., 2020).

Figure 1.1: Asian Clam *(Corbicula fluminea)*

Clams such as *C. fluminea* has always been a part of the local dish, and it is cooked in different ways such as frying, sun-drying, boiled and smoked. However, smoked *C. fluminea* or 'etak salai' is unique in that it is a trendy snack among the local Kelantanese and people living in the southern part of Thailand (Mohamad Shamsul *et al*., 2017; Amir, 2019). Smoked *C. fluminea* is usually prepared by first, removing all muds from the shell surfaces, followed by marinating with spices. It is then smoked on a small fire for a few minutes before it is ready to be consumed (Han *et al*., 2019). Therefore, smoked *C. fluminea* may be considered as ready-to-eat food (Aweng and Maketab, 2006).

Recent studies have shown this edible clam are colonised by different types of bacterial, viral, fungal, and parasites pathogens (Saitoh *et al*., 2007; Ismail *et al*., 2016; Novais *et al*., 2016; Al-Salman, 2017; Bolo *et al*., 2019). A study by Saitoh *et al.* (2007) showed 60% of *C. fluminea* samples from the Gunma prefecture in Japan were found contaminated with Norovirus (NoV) which is known to cause enteric disease in humans. Meanwhile, a protozoan parasite, *Cryptosporidium* is known as one of the common colonisers of *C. fluminea* gut. It causes cryptosporidiosis disease even by ingesting a small number of oocysts (0.1%) contaminated *C. fluminea* which will lead

to abdominal discomfort, vomiting and diarrhoea in infected humans (Bolo *et al*., 2019). Bivalve species could bioaccumulate bacteria several times higher compared to its surrounding aquatic environment, whilst the number of bacterial counts in the gut and flesh of *C. fluminea* is directly proportional and influenced by the microorganisms found in the surrounding environment (Al-Salman, 2017). Many studies have highlighted the ability of *C. fluminea* in accumulating many types of pathogenic microorganisms when growing in polluted sewage environment (Saitoh *et al*., 2007; Ismail *et al*., 2016; Novais et al., 2016; Bolo *et al*., 2019).

Moreover, *C. fluminea* can be a health threat to humans due to its behaviour in harbouring important human pathogens which may cause severe gastrointestinal diseases in humans such as diarrhoea, gastroenteritis, dysentery and salmonellosis. Foodborne diseases can originate from ingestion of contaminated *C. fluminea* into the body by toxic and infectious agents. *Edwardsiella tarda* is an example of a bacteria found colonising in the gut of *C. fluminea* (Wei *et al*., 2013a). This bacterial pathogen can cause diseases in humans and is frequently isolated from blood, urine, faecal, and wounds of patients. Thus, consuming raw or smoked *C. fluminea* may pose a severe threat of foodborne diseases among locals and even curious tourists. Consuming contaminated seafood is a common source of gastrointestinal illness to humans which can lead to severe illness and even death in the young and the elderly. Foodborne bacterial contamination in seafood, sampled from several stalls in east-coast, Malaysia has been reported in several studies (Hamdan *et al*., 2008; Wei *et al*., 2013a; Manan, 2014). Coliform bacteria (*E.coli* and *Klebsiella*) and *Salmonella* spp. were reported presence in the short-necked clams, *Orbiclularia orbiculate* sampled at several stalls in East Coast, Malaysia (Hamdan *et al*., 2008). This short-necked clam was harvested

in areas where *C. fluminea* was also found. Therefore, this study intends to review the microbiological status from the previous study reported from Jun – July 2020.

1.2 Problem Statement

Contamination of aquaculture products by foodborne pathogens has become a significant concern in many developing countries. In Malaysia, fresh seafood and shellfish products contributed 26% of the total outbreaks, and most cases are caused by pathogenic microorganisms (Hamdan *et al.*, 2008). Although bivalves are recognised as an excellent source of protein, however, contamination by pathogenic microorganisms can become a significant health concern. Epidemiologists have identified this problem due to the natural behaviour of bivalve species as a filter-feeder (Khan Chowdhury *et al.*, 2009). The presence of pathogenic microorganisms in *C. fluminea* can make it unsafe for consumption, mainly in the form of raw or ready-to-eat 'etak salai'. *C. fluminea* was chosen in this study because it is one of the ready-to-eat food that is popular amongst the locals in Kelantan. It frequently being sold at the roadside, at the wet market and night market in Bachok, Kota Bharu, and Pasir Mas.

In Kelantan, *C. fluminea* is not harvested from regulated aquaculture farm but mostly obtained from its natural environment. It is usually harvested from the riverbed using basket or clam dredge by the villagers. However, Kelantan River with 248 km long, frequently found to exceed the limits for faecal coliform bacteria set by Interim National Water Quality Standard (INWQS) Malaysia. A recent study by Bong *et al.* (2020) found that this river has exceeded the recommended limits for *E.coli* number permitted by INWQS for class II river. Thus, the pollution of the river by *E. coli* was thought to be the primary sources of microbial contamination. In the meantime,

contamination also can occur due to other reasons, such as improper handling, temperature abuse and, unhygienic practices during food preparation. Thus, its safety for consumption remains doubtful. To date, the availability of information on the microbiological quality and safety of *C. fluminea* in Kelantan is minimal and fragmented.

1.3 Research Objective

General Objective:

To determine the microbiological quality of raw and smoked *C. fluminea* sampled from selected vendors in Pasir Mas, Kota Bharu and Bachok, Kelantan.

Specific Objectives:

i. To determine total plate count and total coliforms counts in raw and smoked *C. fluminea*.
ii. To determine faecal coliforms counts and *E. coli* in raw and smoked *C. fluminea*.

1.4 Significant of Study

In this study, *C. fluminea* from the area of Bachok, Kota Bahru, and Pasir Mas, Kelantan will be sampled and analysed for microbiological quality and safety for human consumption. The information obtained from this research can be used to help in improving the microbiological quality of *C. fluminea*. The information obtained from this study can be used by the local health authorities to formulate a programme for vendors and to inform the public on the safety issue regarding the consumption of raw and smoked local clam, C. *fluminea.*

1.5 Research Conceptual Framework

The research conceptual framework is shown in Figure 1.2. In this study, the raw and smoked *C. fluminea* were sampled using simple convenient sampling. In an over one-month period, thirty samples (n=30) were collected from the street and wet market vendors in the area of Bachok, Kota Bharu, and Pasir Mas. Microbiological quality analyses of the samples were determined from total plate count (TPC) count, total coliform count (TCC) count, faecal coliform count (FCC) count and the absence and/or presence of *Escherichia coli* (EC).

The microbiological quality of smoked *C. fluminea* was based on the *Makmal Kesihatan dan Keselamatan Makanan* (MKKN) Microbiological Guidelines adapted from the Australian Standard Methods for the Microbiological Examination of Food 1993 and Malaysian Food Act 1985. For safe consumption, raw and ready-to-eat smoked *C. fluminea*, the number of TPC should be less than 10 000 per 25g, TCC less than 100 per 25g and less than three colonies per 25 g for a FCC to consider as safe for human consumption.

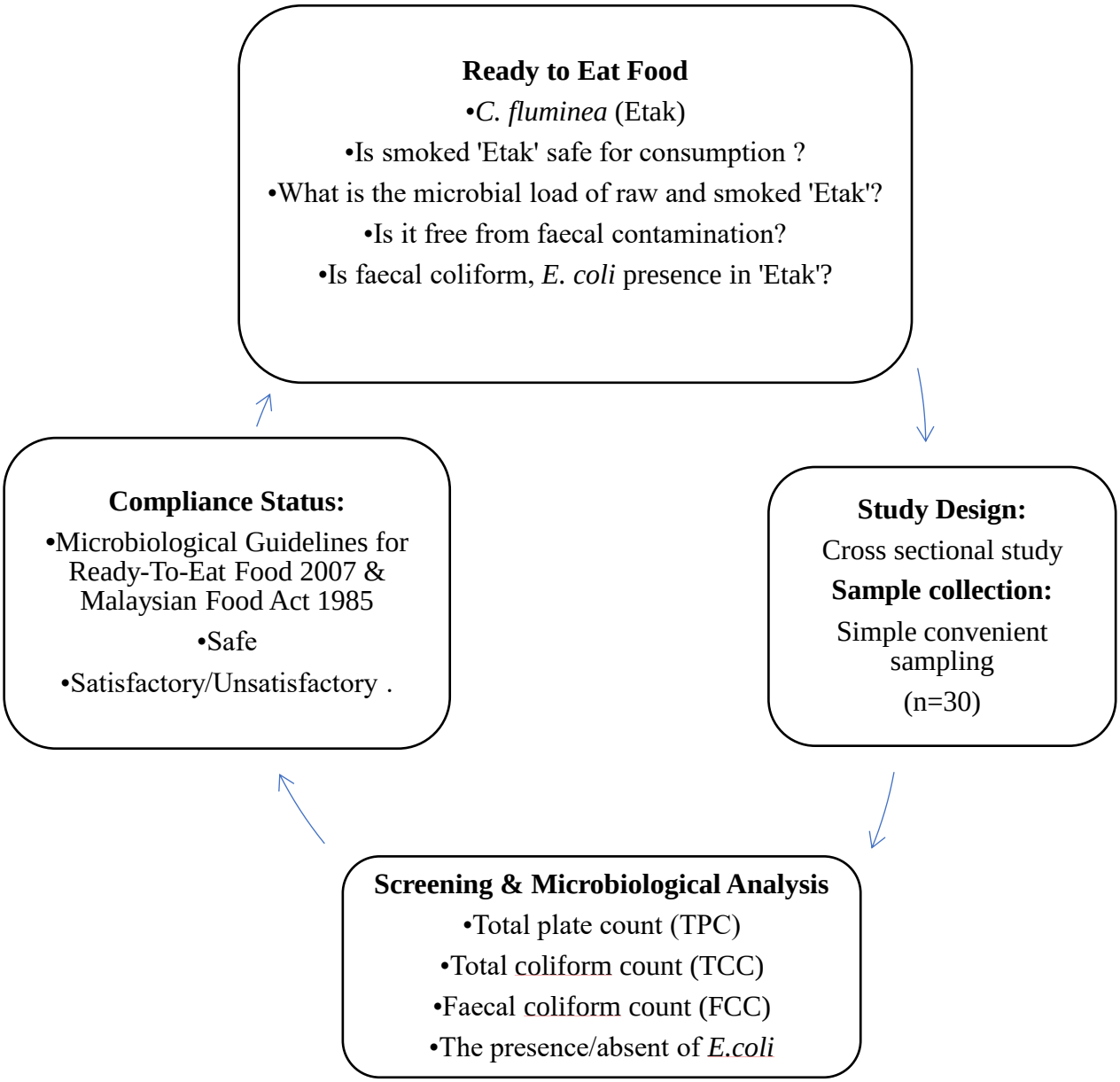


Figure 1.2: Research Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

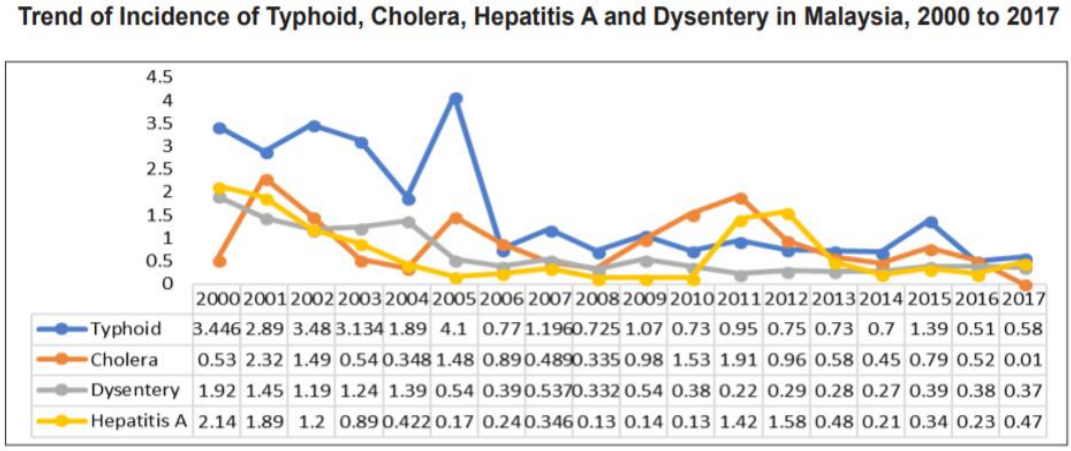
2.1 Foodborne Diseases

Typhoid fever, food poisoning, dysentery, and cholera are several examples of foodborne diseases caused by the contamination of food or water (Table 2.1). These foodborne diseases might be originated from contaminated food by pathogenic microorganisms or excessive chemicals. These pathogenic microorganisms can be transmitted through the faecal-oral route caused by unhygienic behaviour of food handlers, contaminated food (pollution), and unclean environment (Puteh *et al*., 2013). Customers who are unaware of these contaminated foods will become the victim and probably will suffer from the foodborne disease.

The total number of foodborne diseases reported in Malaysia had steadily declined from the year 2012 to 2016. However, in 2015, the incidence rate shows an increasing trend in all diseases (Figure 2.1). Annual Report by the Ministry of Health (2017) showed that most of the foodborne cases happened in the states of Kelantan and Sabah. Most of the people in these states suffered from the lack of sanitary quality of water supply, even though the chlorination program were applied. In 2017, the number of cases for typhoid and cholera showed an increasing trend in the states of Selangor, Kelantan, Sabah, and Wilayah Persekutuan. Failure of food handler to follow the hygienic practices regulated by the government has been identified as the primary cause for the foodborne outbreak in Selangor and Wilayah Persekutuan (MoH Annual Report, 2017).

Table 2.1: Common types of foodborne diseases recorded in Malaysia and their definition.

Type Disease	Case Definition
Typhoid	Typhoid commonly occurs due to the consumption of unclean water supplies and unhygienic facilities, especially contaminated with *Salmonella* Thypi (Annual Report MoH, 2017). Sellers with unhygienic practices also contribute to the occurrence of this illness. This disease can easily be transmitted from the infected patient and carrier (sellers) to the customers.
Cholera	Cholera disease originated from the bacterium *Vibrio Cholera.* Symptoms associated with cholera infection are vomiting and watery diarrhoea. This type of bacteria commonly found contaminated in seafood products such as shellfish (Abdul-Mutalib *et al*., 2015).
Dysentery	Also known as bloody stool caused by inflammatory of the intestine. A patient will suffer from fever, mucus and bloody diarrhoea, and abdominal cramp. Two types of dysentery are Bacillary Dysentery and Amoebic dysentery. Contamination of human faeces in food and drinking water is known to cause this type of disease (Azzimawati, 2011).
Hepatitis A	Several symptoms of Hepatitis A disease are extreme fatigue, acute jaundice, dark urine, and malaise. This disease is caused by Hepatitis A virus (HAV), which affects the liver of consumers when the host ingested contaminated foodstuffs. Patients with Hepatitis A symptoms will be confirmed with an anti-HAV IgM test using serological testing (Puteh *et al*., 2013).
Food Poisoning	Most of foods poisoning cases reported took place in the school canteen and hostels. In 2017, 44.8% of food poisoning cases happened in the Ministry of Education's school. Vomiting and diarrhoea occurrence due to ingestion of food are symptoms of this disease. It appears after 12-72 hours of digestion of contaminated food or drinking water by pathogenic bacteria.



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Typhoid	3.446	2.89	3.48	3.134	1.89	4.1	0.77	1.196	0.725	1.07	0.73	0.95	0.75	0.73	0.7	1.39	0.51	0.58
Cholera	0.53	2.32	1.49	0.54	0.348	1.48	0.89	0.489	0.335	0.98	1.53	1.91	0.96	0.58	0.45	0.79	0.52	0.01
Dysentery	1.92	1.45	1.19	1.24	1.39	0.54	0.39	0.537	0.332	0.54	0.38	0.22	0.29	0.28	0.27	0.39	0.38	0.37
Hepatitis A	2.14	1.89	1.2	0.89	0.422	0.17	0.24	0.346	0.13	0.14	0.13	1.42	1.58	0.48	0.21	0.34	0.23	0.47

Figure 2.1: Incidence rate of foodborne diseases from 2000 to 2017 (MOH, 2017).

Many foodborne diseases in Malaysia are under-reported and undiagnosed. In most time, people will find it more convenient to seek medication at the private clinic or pharmacy rather than waiting hours for treatment at the government hospital. The number of cases reported might be the tip of an iceberg since many incidences happened were not reported. However, most cases reported are from incidence, which happened at schools (Lim, 2002). According to MOH, an outbreak can be clarified when two or more cases happened to the consumer with the same symptom due to the ingestion of a common food source (Abdul-Mutalib *et al.*, 2015).

In underdeveloped countries, foodborne diseases are still a significant health problem for the citizen. Several causes of foodborne diseases are beyond their control, such as short of clean water, unsanitary environment, and lack of information to food safety (Salleh *et al.*, 2017). This situation becomes worse for vulnerable people such as children below 5-year-old, pregnant women, elderly people and an immunocompromised person who have a high risk of being affected by foodborne diseases (Oliveira *et al.*, 2011).

Foodborne diseases also can cause some economic loss since patients who affected will seek treatment and need to spend money on medical expenses. In severe

cases, patients will be hospitalized and need to absent from work. If an outbreak was reported, government agencies need to do an investigation, and this will become indirect expenses for them. Even worse, the public will become less interested in outside food due to the frequently reported cases. This situation will make people more careful about buying food outsides and indirectly will affect the business involved.

Besides that, most of the local or traditional food seller sometimes neglecting the sanitary of food premise during food preparation. Thus, the safety of food for consumer consumption can be compromised. Each local seller has a traditional recipe for food preparation, and some cooking procedures might be inappropriate in inhibit the microorganism present. Microbial contamination can happen when no safety and precaution is taken before, during and after food preparation. Foods affected by pathogenic microorganisms also will have shorter shelf-life and will reduce its quality at the same time (Aziman *et al*., 2014).

Most of the roadside hawkers are unlicensed, and they frequently found preparing their foods in a private home, and this will make it difficult for local enforcement to do surveillance. In worst cases, if the person who engaged with food preparation failed in receiving typhoid vaccine, they have a possibility to spread the *Salmonella* Typhi bacteria to others. Even though the patient infected has recovery from this type of disease, they still become a carrier for the pathogenic bacterium which only lives in the human unless they are medically certified free by the physician (Infomed, 2015). In Malaysia, food handlers are required to get a typhoid vaccination as one pre-requirement to make them licensed for food handlers (Lum, 2019).

In most cases, the foodborne disease can cause mortality to the patient if the victim is late in seeking medical treatment (Abdul-Mutalib *et al*., 2012). In total, the

number of mortalities recorded in Malaysia was declined from the year 2015 with 11 cases to 10 cases in 2017 (Annual Report MoH, 2017). The number of mortalities for diarrhoea was 3% of the total population of the world, and this was the primary cause of mortality for less developed countries (WHO, 2015). Diarrhoea is characterized mainly by dehydration and acidosis caused by excessive body fluid and electrolyte deterioration (Ayulo *et al*., 1994).

In several studies, the origins of the pollution known to came from the area where it originated (river, pond, wet market). However, several factors also can affect the sanitary of food products. For example, poor quality of raw material, unhygienic practices by food handlers, unclean tools, and utensils are several common reasons reported for food contamination. Bacteria, fungi, viruses, and parasites always linked to foodborne diseases, and these microorganisms can multiply conveniently in the human gut flora.

2.2 Foodborne Pathogens

Microorganisms such as parasites, fungi, viruses, and bacteria are known etiologic agents for foodborne diseases. Most of the food contamination did not visualise any physical changes to the foods matrix and even producing unpleasant odour (Abdul-Mutalib *et al*., 2012). Pathogenic microorganisms can be found in contaminated food and water when sanitary is not prioritised. Several factors have been recognised to be the causes such as cross-contamination, temperature abuse or unhygienic practices during harvesting, processing, transportation, and storage (Aziman *et al*., 2014).

Pathogenic microorganisms can contaminate food at different processing stages, and they can implicate foodborne disease in most cases. With the current trend and urban lifestyle, increasing demand for street food and ready-to-eat food has increased the opportunity of foodborne diseases to occur. For example, most fruits, vegetables, and ready-to-eat foods undergo poor sanitation treatment before entering the market. These unhygienic practices may lead to health hazards to the consumer, and if no proper and hygienic practices are implemented.

Kadariya *et al.*, (2014) and Soon *et al.*, (2011) reported Novovirus, non-typhoidal *Salmonella* spp., *Clostridium perfriengens* and *Campylobacter* spp. are the most reported microorganisms cause for foodborne diseases in the United States. Due to these microorganisms, 3000 people died, and 128 000 people have been hospitalised annually in the United States (Law *et al*., 2015). In Malaysia, the number of cases for food and waterborne diseases varies every year. In 2017, typhoid and hepatitis A diseases showed an increasing trend (Figure 2.1). However, cholera and dysentery recorded a downward incidence case (Annual Report MoH, 2017). Nowadays, local

sellers and food suppliers should abide by the rules and Standard Operating Procedure (SOP) regulated by the Malaysian government to prevent the incidence from recurring in the future.

Table 2.2: Common foodborne microorganisms and their clinical symptoms (Oliveira *et al*., 2011; Suleiman *et al*., 2017).

Microorganism	Pathogen	Symptoms
Bacteria	*Salmonella* spp.	Fever along with gastroenteritis, abdominal cramps and diarrhoea. Body temperature can increase to 39°C to 40°C.
	Shigella spp.	Severe dysentery, abdominal pain, high fever and vomiting.
	Campylobacter jejuni	Diarrhoea (commonly bloody), vomiting, nausea and abdominal cramp.
	Enterotoxigenic *E. coli* (ETEC)	Suddenly watery but non-bloody diarrhoea, abdominal pain, vomiting and mild or no fever.
	Vibrio cholera	Vomiting and acute watery diarrhoea.
Viruses	Norovirus (NoV)	Diarrhoea, abdominal cramp, nausea, fever, and headache
	Hepatitis A virus (HAV)	Dark urine, jaundice, diarrhoea, and flu-like symptoms
	Enterovirus	Vomiting, malaise, headache, fever
Parasites	*Cryptosporidium* spp.	Abdominal discomfort, vomiting and mild fever, watery diarrhoea
	Giardia lamblia	Bloating, acute or chronic diarrhoea, flatulence
Algae (Intoxication)	Biotoxin secreted by Dinoflagellates genus of *Pyrodinium*	Asphyxiation, tingling sensation of the lips, mouth, and tongue, dizziness; vomiting, nausea

2.2.1 Bacteria

Enterobacteriaceae species are a group of Gram-negative bacteria that frequently found to be an opportunistic pathogen for foodborne disease (Estuningsih *et al*., 2006). The hot and humid temperature throughout the year make Malaysia a suitable place for bacteria to grow on foods. There are several pathogenic microorganisms known to be causative for foodborne diseases. In the United States, the most common causes of foodborne illnesses are due to bacterial pathogens (76%), followed by viruses (21%) and parasites (2.6%) (Law *et al*., 2015). In South-East Asia, Norovirus, pathogenic *E. coli,* and non-typhoidal *Salmonella* spp. are known to be the most common causes of foodborne diseases, especially to the vulnerable group. Older adults, children below five years old, and pregnant women are several groups of people who are profoundly affected by foodborne pathogens (Salleh *et al*., 2017).

Coliform bacteria usually used as an indicator of microbial contamination in food and water samples. It can be grouped as Gram-negative bacteria that can ferments lactose within 48 hours at 35°C, to produce gas and acid during the incubation period (Hamdan *et al*., 2008). Faecal contamination can be assumed by the presence of these types of bacteria, which can give potential hazardous to the infected host. A watery system that polluted with untreated sewage pollution is known to be the origin of coliform bacteria and becomes the primary source for food contamination. Prevalence of *E. coli* isolated in stools has been reported in many studies and contributed to most outbreaks. One technique that has been commonly used internationally in assessing potential risks towards the consumer is the enumeration of total coliform, faecal coliform, and detection of *E.coli* (Nordin *et al*., 2011). The Environment Protection

Agency (EPA) uses the enumeration of fecel coliform bacteria in water as their guidelines to determine the standard of water sanitary (Brock, 2003).

E. coli is a commensals microorganism found to be present in the healthy gut flora of human and animal. It is harmless under normal circumstances, as long as no virulence genes are present inside the bacteria associated with enteric infection. Specific serotypes of pathogenic *E. coli* have been implicated in several outbreaks. They are commonly associated with diarrheagenic. These serotypes also can be transmitted from animals to humans, mostly through cross-contamination via water and foods. Pathogenic *E. coli* can be classified into five different classes based on specific virulence genes presence inside the bacterium, Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Enterohemorrhagic *E. coli* (EHEC) and Enteroaggregative *E. coli* (EAEC) (Evans & Evans, 1996). Some ETEC types produce a toxin called cytotoxic, causing mucosal cell of an infected person to be damaged and cytotonic toxin responsible for the secretion of water and electrolytes in a patient. This type of serotype is always linked to diarrhoea illness among traveller and new-born babies in developing countries. Common symptoms of ETEC infections are suddenly watery but non-bloody diarrhoea, abdominal pain, nausea, vomiting and mild or no fever. EHEC is characterised by symptom of bloody diarrhoea on patient caused by single serotype (O157: H7). EHEC serotype is also known to cause haemorrhagic uremic syndrome (HUS) and haemorrhagic colitis (HC) which responsible for high morbidity and mortality commonly in children. Infantile diarrhoea in children usually associated with EPEC responsible for 1.3 million death every year in children below five years old globally (Ochoa and Contreras, 2011).

As a sub-group of coliform bacteria (Figure 2.2), *E. coli* commonly used as a routine hygienic indicator in assessing the quality of food and drinking water. However, when certain specific diseases are reported, or the indicator bacteria levels are high, the specific pathogenic test will be conducted. In most of the time, pathogenic microorganisms present at the lowest number in the contaminated water or food. The faecal coliform count is much more appropriate in determining the contamination of water and food with human faeces. Other microorganisms used as a safety indicator are enterococci, faecal coliform and coliphages. Several faecal coliform indicators commonly are *Campylobacter* spp, *Staphylococcus aureus*, *Shigella* spp., (C.W., 2020).

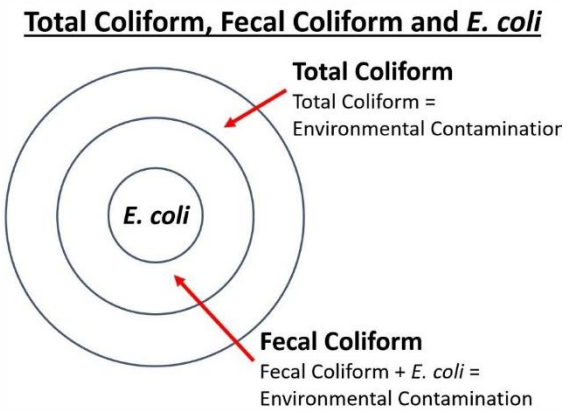


Figure 2.2: Relation between TC bacteria, FC and an indicator *E.coli* (MOH,2017).

A serotype of *Salmonella* spp, *Salmonella enterica* found to affected people worldwide by the diarrhoeal disease, which caused 230 000 deaths annually. It becomes the second most significant foodborne disease in the world. This type of pathogenic bacteria remains consistent and contributed highest for morbidity and mortality rates among foodborne illnesses (Salleh *et al*., 2017). Seafood product is one of commonly reported to be a vehicle for transporting this type of pathogen to human. Its ability to survive in a different type of condition has become a leading cause of

foodborne illness, including in processing and production chains. Nausea, fever, and vomiting are other symptoms by *Salmonella* spp. These symptoms may last for several days, depending on the strain of bacteria-infected, the volume of contaminated food consumed and individual host tolerance. Delay in receiving treatment can cause death to the patient. In the year 2002, 604 000 people in South East Asian recorded to be affected by the diarrheal disease *(*Ngiam *et al*., 2020).

The third most common bacteria group to be implicated in diarrhoeal diseases in children is *Shigella* spp., and they have become a health problem in Malaysia. *Shigella dysenteriae* is an enteric bacterium known to cause bacillary dysentery in human. Effectively even in low dose, the disease is usually caused by as little as 10–100 bacilli since it can easily survive gastric acidity than other enterobacteria. The bacilli invade and multiply the villi's epithelial cells within the large intestine, spread laterally to neighbouring cells, and penetrate the lamina propria. Quick treatment using an anti-microbial agent can inhibit its virulency, and will stop their transmission to other.cells (Banga Singh *et al*., 2011).

Since 1980, *Campylobacter* has been known to cause illness in humans. Campylobacteriosis is responsible for the cause of over 100 deaths in the United States, and most cases are sporadic. Contaminated drinking water, chicken products and raw milk are known sources to implicated campylobacteriosis. Its complication includes diarrhoea (commonly bloody), vomiting, nausea and abdominal cramp (Silva *et al*., 2011).

Vibrio cholera, Vibrio parahemolyticus and *Vibrio vulnificus* are several pathogenic Gram-negative bacteria are known to be natural inhabitants in the coastal marine area and frequently found in shellfish products. The genus *Vibro* spp., are

prevalent in the tropic zone throughout the year and only can be detected during the hot season in a temperate area. Cholera disease caused by El Tor O1 *V. cholerae* infected the human intestine causing symptoms of vomiting and acute watery diarrhoea (Teh *et al*., 2012).

2.2.2 Viruses

Recently several gastrointestinal outbreaks have been connected with contamination of foodstuff with viruses. Norovirus (NoV) and Hepatitis A virus (HAV) are the most common viruses causing human gastroenteritis in the United States and other developing countries. Diarrhoea, abdominal cramp, nausea, fever, and headache are the typical clinical symptoms suffered by humans infected with NoV. This non-bacterial pathogen is known to make shellfish as the reservoir due to the shellfish's behaviour as a filter-feeder (Elbashir *et al*., 2018). Transmission rates will be higher when people are located in a closed setting area. In the same time, the outbreaks will persist for several months if no immediate action is taken.

Other than resistance to freezing, NoV is also known to be resistant to commercial disinfection. This virus frequently presents in shellfish and can survive for an extended period in the aquatic environment. The autoclaving process is one technique known to be an effective way to inhibit the growth of this microorganism (Elbashir *et al*., 2018). NoV outbreak has never been reported in Malaysia until now due to underreporting nor no diagnostic facilities readily available in this country (Subahir *et al*., 2019).

HAV is a minor concern and cause acute illness that is due to the ingestion of faecal contaminated food, improper handling, and unhygienic practices. A patient who

infected with HAV shows various conditions such as mild in most cases and gives severe condition, especially towards pregnant women, immunocompromised people, and the elderly. Patients who are infected with HAV will have signs such as dark urine, jaundice, diarrhoea, and flu-like symptoms (Oliveira *et al*., 2011). This type of disease become a reportable disease in Malaysia since 1988. Nevertheless, the number of cases reported has decreased since, due to the implementation of a control program conducted by the Malaysian government (Raihan, 2016). Contaminated shellfish consumption was the frequent cases reported in HAV foodborne outbreaks (Yusoff *et al*., 2015). This outbreaks frequently prolonged for several days, and the quarantine procedure was an effective way implemented to control the outbreaks (Subahir *et al*., 2019).

2.2.3 Protozoan Parasites

Studies regarding protozoan parasites on bivalve species in Malaysia are scarce. Shellfish tend to be infected with protozoan parasites from the surrounding environment and also can become a host carrier. *Cryptosporidium* spp. causes cryptosporidiosis in humans and animals. It is mainly ingested via contaminated food and water and caused abdominal discomfort, vomiting, and mild fever to the patient. *Cryptosporidium* spp. will spread into the new host by subsequent shedding even at a low-infectious dose (10-30 oocysts). This pathogenic protozoan also has the ability to infect immunocompetent persons. Annually, 58 million cases were reported worldwide on intestinal protozoan infection caused by this parasite in children (Putignani and Menichella, 2010).

Giardia lamblia is another protozoan parasite known to causes gastrointestinal illness in humans. Giardiasis disease is among the highest parasite causative illness recorded in several countries such as Australia, North America, and Scotland. It can transmit into the new host through contaminated water (oocysts) by penetrating through human skin during water activity (bathing, swimming, diving), or accidentally ingested through contaminated food or water. Protozoan parasites can withstand a harsh environment and resistance to chemical disinfection (chlorination) due to *Cryptosporidium* oocyst and *Giardia* cyst transmissive stages. A histopathological survey using a microscope is a commonly used technique to examine and diagnose of protozoan parasites contaminated in shellfish tissue (Kumar *et al*., 2014).

According to Kumar *et al*. (2014), studied on parasites contamination in water were started in 2004 in Malaysia. Since that, *Cryptosporidium* and *Giardia* were detected in different water environments such as sewage samples, recreational lakes, and rivers. In Philippine, bivalve species sampled from Marina Bay were found to be contaminated with *Cryptosporidium* spp. This contamination usually originated from wild and domestic animal waste that inhabit in the surrounding area (Bolo *et al*., 2019). *Entamoeba histolytica* is another protozoan parasite known to cause amoebic dysentery in humans. However, in South East Asia countries, studies on detection of parasites are more likely conducted on animals compared detection directly from the water sample.

2.2.4 Algae

Filter feeding bivalves such as clam, mussels, and oysters used planktonic algae as the source of their main foods. The occurrence of harmful algal blooms (HABs) due to changes in environmental conditions can indirectly cause

gastrointestinal illness and neurological disease on the consumer by consuming alga contaminated bivalves. This disease can infect people when the uptake of shellfish contaminate with HABs (Noor *et al*., 2007). In Malaysia, the most reported HAB related shellfish poisoning case was paralytic shellfish poisoning (PSP) (Usup *et al*., 2002). Saxitoxins (STXs) are fast-acting neurotoxins that responsible for illness on the human who ingested contaminated seafood. *Pryodinium, Alexandrium* and *Gymnodium* are several dinoflagellates genera found to be able to produce these toxins in marine water.

Humans who ingested shellfish contaminated with STXs will undergo PSP symptoms within 30 minutes. If no further treatment is taken, asphyxiation will happen to cause fatality to the patient. Almost 2000 cases were reported worldwide every year on PSP outbreaks, which can have adverse effects on economics, tourism, and the aquaculture industry. In Malaysia, HABs or red tides phenomenon commonly happen, and the first outbreak was recorded in 1976. The latest case reported happened in Kota Kinabalu, Sabah, when 58 people undergo PSP symptoms and cause four death due to the consumption of contaminated shellfish with STXs (Suleiman *et al*., 2017).

2.3 Food Safety and Ready-To-Eat Food

All food products in Malaysia are under strict compliance with the Malaysian Food Act, 1983 and Food Regulation, 1985. Quality control for all foods safety in Malaysia is under the purview of the Ministry of Health, and they must comply with the Food Act 1987. In most developed countries, surveillance authorities have been introduced to monitor food safety and the occurrence of a foodborne outbreak. When an outbreak occurred, a notification will be sent by the physician in public health care

facilities to the Disease Control Division (DCD), Ministry of Health. Five different types of food and water diseases required to be reported under the Prevention and Control of Infectious Disease Act 1988, as mentioned in Table 2.1.

In 2008, Malaysian seafood products were banned from entering the European Union (EU) countries. It was due to the quality of the products that failed to meet the minimum standard quality sets by EU's agriculture audit authorities. Government of Malaysia suffered RM600 million losses in export value due to the sanction (Retnam *et al*., 2013). Microbiological testing is indispensable in determining the quality and safety of food products for human consumption. This testing is to ensure compliance with the regulatory requirement stated, in preventing food poisoning outbreaks from happens. Hazard Analysis Critical Control Point (HACCP) has been introduced as a quality assurance (preventive system) in many food industries worldwide. It plays a vital role in protecting the consumer from hazardous foods. Microbiological tests in the food industry have two main objectives. First, to ascertain either pathogenic microbes or their toxin presence in the food due to contamination (food safety), and to estimate the microbial load in determining the effectiveness of hygienic process (food quality) (Umesha and Manukumar, 2018).

Due to the urban lifestyle and time consuming to food preparation, ready-to-food (RTE) has significantly become an option, especially for people with a hectic lifestyle. RTE food usually prepared hours before it ready to be sold to the consumer. During the selling period, RTE food will be displayed without cover and this increase the risk of cross-contamination from the open environment. Open food outlet and roadside stalls might be a popular place for Malaysian to have their food. However, consumers are not aware of pollution coming from the surrounding area, especially dust and smoke emission due to vehicles passing by (Salleh *et al*., 2017).

Cleanliness of food outlets also contributed to the occurrence of a foodborne outbreak. Cleanliness of food premises is under the purview of the local authorities with cooperation with the local Health Department. In this matter, local authorities will grade the food premise either as A (good), B (moderate and caution) or C (poor with closure order) (Ali and Abdullah, 2012). The purpose of this graded is to alert the customer with the cleanliness of the premise. However, most Malaysian prefer to patron premises with low price food instead of the hygienic food premise.

According to Puteh *et al.*, (2013), the occurrence of diarrhoea outbreak among the ethnic Chinese in Malaysia is low, due routine in having the meal when it still hot or immediately after the preparation. This habit will be lowering the risk of food to be contaminated by pathogenic microorganism. Several factors also have been known as the contributor to the contamination of RTE food. Unhygienic practices, poor environment, exposure to open environment, unclean water source and temperature abuse are several factors that contributed to foodborne outbreaks.

2.4 'Etak' (*C. fluminea*)

Aquatic animals are an essential source of protein for human consumption, and it becomes popular in the recent year. Bivalve mollusc has significantly increased in demand, but contamination of this gourmet food with pathogenic microorganism still become a significant problem in the aquaculture industry. The accumulation of pathogenic microorganism in bivalve is due to its natural habit of pedal-feeding during the food uptake. As a filter feeder, bivalve tends to concentrate particulate matters from their surrounding environment, including pathogenic microorganisms. A study by (Khan Chowdhury *et al.*, 2009) showed bivalve species such as mollusc clam and