ANALYSIS OF TOTAL COLIFORM AND *ESCHERICHIA COLI* (*E. Coli*) IN FRESHWATER AND MARINE BIVALVES.

by

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LIST OF ABBREVIATION

FAO	Food and Agriculture Organization of the United Nations
DOF	Department of Fisheries
USA	United State of America
COVIS	Cholera and Other Vibrios Illness Surveillance System
DST	Diarrheic Shellfish Toxins
DSP	Diarhheic Shellfish Poisoning
CFU	Colony forming unit
MPN	Most probable number
NHMRC	National Health and Medical Research Council of Australia
APHA	American Public Health Association
EPEC	Enteropathogenic *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EAEC	Enteroaggregative *E. coli*
ETEC	Enterotoxigenic *E. coli*
DAEC	Diffusely adherent *E. coli*
HC	Haemorrhagic colitis
HUS	Hemolytic uremic syndrome
VRB	Violet red bile
SPC	Standard plate count
AOAC	Association of Analytical Communities
TNTC	Too numerous too count
SOP	Standard of procedure
HAV	Hepatitis A virus
NoV	Norovirus

ANALISIS JUMLAH KOLIFOM DAN *ESCHERICHIA COLI* (*E. Coli*) DALAM DWICANGKERANG AIR TAWAR DAN AIR MASIN.

ABSTRAK

Dwicangkerang sering dikaitkan dengan insiden penyakit bawaan makanan. Jumlah kolifom dan *E. coli* sering digunakan sebagai penentu mikrobiologi untuk menentukan tahap pencemaran najis dalam dwicangkerang dan status kualiti air. Objektif kajian ini adalah menganalisis kehadiran jumlah kolifom dan *E. coli* dalam dwicangkerang air tawar dan air masin terpilih. Dalam kajian ini, dua jenis dwicangkerang air tawar: kerang darah (*Anadara granosa*) dan kerang Asia (*Corbicula fluminea*) telah dipilih manakala dwicangkerang air masin yang terlibat ialah kerang karpet (*Paphia textile*) dan tiram (*Crassostrea* spp.). Analisis mikrobiologi telah dijalankan melalui ujian pantas konvensional dengan menggunakan 3M Petrifilm *E. coli*/Coliform Count Plate. Semua sampel dianalisis dalam bentuk mentah ataupun segar dan dimasak dengan julat masa yang berbeza. Keputusan analisis menunjukkan semua sampel mentah dicemari dengan jumlah kolifom dan *E. coli*. Jumlah kolifom dan *E. coli* dikesan dalam dwicangkerang yang dimasak dalam masa singkat (masa memasak 5 dan 10 minit) tetapi tidak bagi dwicangkerang yang dimasak sepenuhnya (masa memasak 15 dan 20 minit) kecuali A*nadara granosa* dan *Paphia textile*. *Corbicula fluminea* telah dikenalpasti sebagai dwicangkerang paling tercemar jika dibandingkan dengan sampel lain. Kesimpulannya, dwicangkerang mentah dan dimasak dalam tempoh singkat tercemar dengan bakteria patogen dan tidak selamat untuk dimakan oleh manusia.

ANALYSIS OF TOTAL COLIFORM AND *ESCHERICHIA COLI* (*E. Coli*) IN FRESHWATER AND MARINE BIVALVES

ABSTRACT

Bivalve molluscs commonly associated with many foodborne disease outbreaks. Total coliform and *E. coli* are always being used as microbial indicator to determine fecal pollution level in bivalves and water quality status. The objective of this study is to analyze the presence of total coliform and *Escherichia coli* in selected freshwater bivalves and marine bivalves. In this study, two freshwater bivalves: blood cockle (*Anadara granosa*) and Asian clam (*Corbicula fluminea*) were selected while two marine bivalves involved in this study were oysters (*Crassostrea* spp.) and carpet clam (*Paphia textile*). Microbiological analyses were conducted through conventional rapid test method by using 3M Petrifilm *E. coli*/Coliform Count Plate. All samples were analyzed in term of raw or fresh sample and cooked with different time interval. Results of these analyses showed that all raw samples were contaminated with total coliform and *E. coli*. Total coliform and *E. coli* were also found in lightly cooked bivalves (5 and 10 minutes cooking time) and not in fully cooked bivalves (15 and 20 minutes cooking time) except for *Anadara granosa* and *Paphia textile*. *Corbicula fluminea* identified as most contaminated bivalves as compared to other samples. In conclusion, raw and lightly cooked bivalves are highly contaminated with pathogenic bacteria and unsafe for human consumption. In order to reduce and eliminate number of bacteria, bivalves must be cooked thoroughly with proper cooking temperature and method.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Bivalve is one of subclass Phylum Molluscs under animal kingdom, which consists approximately 7500 species. Gosling (2003) has defined bivalves as animals with two shell valves and divided from front to back into left and right valves. Oysters, scallops, mussels, clams and cockles are general groups of bivalve that normally consumed by human. Shell and mantle inside the bivalve cavity have been used to differentiate various species of bivalves. This includes shell's colour, size and shape. Basically, bivalves come from small particle size, which is about one millimetre (0.04 inch) in length and can be up to more than 137 centimetres (54 inches). It also can weigh up to 264 kilograms (582 pounds) especially for giant clam that occupied in South Pacific coral reefs.

Ecology of bivalves is huge and wide while different regions consist of different species of bivalves. Different physical and environmental characteristics in each region such as temperature, salinity, seasonal influence and light are the reasons of bivalve's diversity (Gosling, 2003). As an example, *Mytillus californianus* mussels are highly occupied at the Pacific Ocean costal in North America while *Perna viridis* mussels can be found easily in Indian Ocean and Southeast Asian region. This diversity also is applicable to other species of bivalves.

Oyster species *Saccostrea cucullata* occupied at the Indian Ocean costal region from India up to South Africa and difficult to be found in other region (Gosling, 2003). Southeast Asia is reported to have 1211 species of molluscs. Therefore, Southeast Asia region has been recognised as most dense region with bivalves species as compared to other regions around the world such as Europe, China and United State of America (Crame, 2000).

In Malaysia, several species of bivalves are being harvested and cultivated for commercialisation such as molluscs arch clam (*Orbicularia orbiculata*), blood cockles (*Anadara granosa*), oysters (*Crassostrea* spp.) and Asian green mussels (*Perna viridis*) (FAO, 2004). These bivalve species are normally consumed by Asian population generally and Malaysian specifically. In fact, Sarawak region only has almost 15 bivalves species that have been recorded and detected (Hadi *et al.,* 2012). Most of these bivalve species can be found also in Peninsular Malaysia. Besides that, bivalves have been consumed in well-cooked form among Malaysian and eating raw or lightly cooked are still commonly practiced due to bivalve's juiciness.

Shell of bivalves is made up with high concentration of calcium carbonate under biomineralisation process that embedded in an organic matrix (Jacob *et al.,* 2009). Shell has been considered as an important part in bivalves because it acts as a skeleton to hold bivalves' flesh and defend mechanism from predators. Mantle cavity is also one of the important organs in bivalves. Component and flesh of bivalves in mantle cavity helps in differentiating various species of bivalves. Furthermore, muscle or flesh of bivalves is important for locomotion, which muscles in bivalves are able to retract the foot and effect back-and-forth movements.

1.2 Rationale of study

Bivalves have unique feeding mode and characteristics. Bivalves are normally undergoing filter feeding or suspension method to gain nutritious compounds. Through this feeding mechanism, suspended solids that contain organic and inorganic matter always being entrapped in bivalves cavity and gill. Concentration of suspended solids will give some effect to organism itself such as increase size of the species population, reduced flesh density and clogging in gills or gut (Bilotta & Brazier, 2008).

Clogging and suspended materials are highly condensed with many pathogenic bacteria, viruses and plankton. Therefore, most of filter feeder organisms are carrier to infectious diseases. Transmission of pathogenic microorganisms may occur through bivalve consumption in food. Strong evidence based on several food-borne disease outbreaks showed that pathogenic bacteria and viruses tend to accumulate in bivalve and cause illnesses such as diarrheas, vomiting and gastroenteritis (Hernroth *et al.,* 2010; Potasman *et al.*, 2002a; Rehnstam-Holm & Hernroth, 2005).

Worldwide studies have suggested that bivalves can be a vehicle for pathogenic bacteria such as *Escherichia coli* (*E. coli*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Vibrio cholera* (*V. cholera*), *Salmonella* spp. and *Shwenella putrifaciens* (Najiah *et al.*, 2008; Rehnstam-Holm & Hernroth, 2005). Study by Hatha *et al.* (2005) on bivalve clam *Batissa violeca* at Suva Market in Fiji proved that various genera of heterotrophic bacteria are detected such as *Microccocus*, *Bacillus*, *Acinobacter*, *Vibrio*, *Aeromonas*, *Alcaligenes*, *Pseudomonas*, *Streptococcus* and Enterobacteriaceae.

Production and consumption of mollusc bivalves species in worldwide constantly increased from year to year. FAO reported that oysters production has increased from 2013 to first 9 month of 2014. Amount of international trade for oysters have reached 38 1000 tonnes with USA as major importer. Meanwhile, China has been recognized as world's largest scallop trader with 30% of total world import and 32% of world exports (FAO, 2015). However, production of mussels from European countries dropped from 750 000 tonnes in the late 1900s to 200 000 tonnes in past few years and drop rate is approximately 500 000 tonnes. This reduction occurred due to environmental factors such as poor water quality, pollution, biotoxins and finding spaces for future sites (Monfort, 2014).

In Malaysia, production of bivalves is monitored by Department of Fisheries, an agency under Ministry of Agriculture and Agro-Based Industry. Blood cockles, clams and oysters are major bivalves species that can be found and culture in Malaysia. During 2012, total production of cockles is 42 132.03 tonnes. This amount has contributed RM 60.01 million per year to country income. Kedah, Penang, Perak, Selangor and Johor have been listed as main producer for cockles to entire Malaysia with 389.97, 7737.34, 22068.56, 11842.66 and 93.50 tonnes respectively (Dept. of Fisheries Malaysia, 2012). This statistical data proved that Malaysia also has took part progressively in the production and supply of bivalves for consumers.

From 1969 to 2000, several outbreaks related to bivalve consumption have been identified and most of the outbreaks happened due to consumption of raw or lightly cooked bivalve. Regarding all reported outbreaks, shellfish and bivalve contributed 64% of total seafood-associated outbreaks in the world in conjunction with pathogenic bacterial and viruses contamination (Oliveira *et al.*, 2011; Potasman *et al.*, 2002b). Therefore, study and analysis regarding occurrence of pathogenic bacteria in bivalve should be conducted frequently to ensure bivalve that produced are safe to be consumed.

Primarily, the objective of this study is to analyze and enumerate microbial indicator: total Coliform and *Escherichia coli* (*E. coli*) in bivalves that commonly consumed in Malaysia generally and state of Kelantan specifically. In this study, four types of freshwater and marine bivalves have been selected. Freshwater bivalves are 'kerang' or blood cockles (*Anadara granosa*) and 'etok' or Asian clam (*Corbicula fluminea*), while marine bivalves are known as 'lala' Carpet clam (*Paphia textile*) and 'tiram' Oysters (*Crassostrea* spp.). These species are being selected as sample is due to their availability in research setting.

As example, 'etok' are very popular and easily found in state of Kelantan. 'Etok' have been smoked by using several herbs and spices. "Etok salai" normally sold at stall along street or night market. In fact, 'etok salai' is popularly consumed among Kelantanese as snack instead of main dish during meal. There are only a few studies concerning microbiological quality in bivalves done in Malaysia, Therefore, study of microbiological quality of mostly consumed bivalve in Malaysia is advisable. In addition, this study is beneficial to provide microbiological data in food safety.

Furthermore, most of the study in bivalve microbiological quality has been done on detecting viruses and specific type of bacteria such as *Vibrio* species and *Salmonella*. (Najiah *et al.*, 2008). Meanwhile, majority of samples that involved in previous study are collected from harvesting and cultivating center instead of commercial setting such as wet market and hypermarket. Besides that, only few studies regarding microbiological quality of bivalves that could be found in Kelantan specifically.

This study is important because microbial component in bivalves highly influenced by several factors such as environmental factors: climate, temperature and seasonal, method of handling, storing and also urbanization (Campos & Cachola, 2007; Hernroth *et al.*, 2010). Therefore, results that will be obtained in this study could help to determine safety level of four most consumed bivalves in this population. Other than that, results in this study could be used as guideline in determining and monitoring of water quality in bivalves habitat for future analysis.

1.3 Objectives of study

1.3.1 General objective

i. To analyze total coliform and *Escherichia coli* (*E. coli*) in selected freshwater and marine bivalves.

1.3.2 Specific objectives

i. To calculate the microbial load of total coliform and *E. coli* in blood cockles (*Anadara granosa*), Asian clams (*Corbicula fluminea*), carpet clams (*Paphia textile*) and oysters (*Crassostrea spp.*)

ii. To analyze the pattern of total coliform and *E. coli* load in raw bivalves and cooked bivalves.

iii. To analyze the pattern of total coliform and *E. coli* load in different serial dilution.

CHAPTER 2

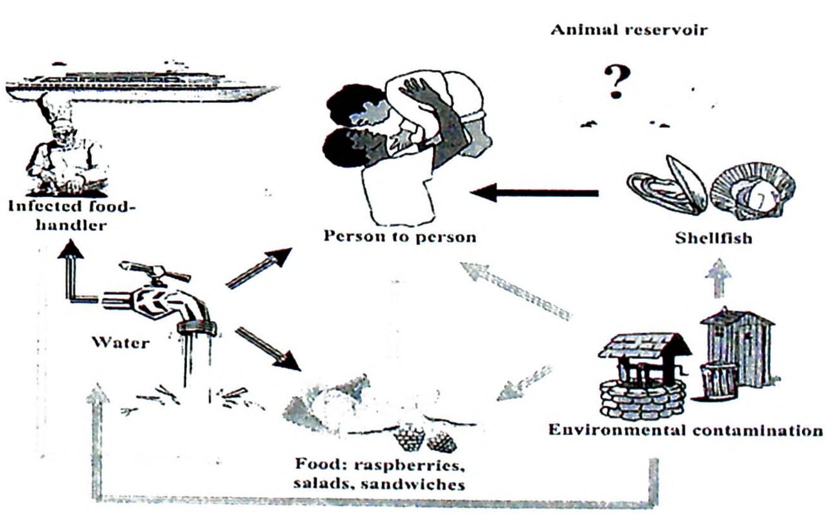
LITERATURE REVIEW

2.1 Foodborne diseases and bivalves contamination.

Ingestion and consumption of raw and lightly cooked bivalves are highly associated with many foodborne disease outbreaks. Foodborne disease outbreaks can be defined as when two or more individuals experience similar symptoms and illness due to ingestion of common foods. This foodborne outbreak occurred caused by ingestion of contaminated bivalves (Pereira *et al.*, 2006). Contamination of bivalves majorly contributed by accumulation of enteric bacteria and viruses from the environment (Roldan *et al.*, 2011; Potasman, Paz, & Odeh, 2002; Prato *et al.*, 2013).

In determining contamination level of bivalves, total coliform and E. coli have been used as contamination indicator. In addition, *E. coli* attributed more than 85% of fecal coliform concentration. Fecal coliform can infect human due to fecal-oral route and pathway through ingestion of contaminated bivalve (Jalal *et al.*, 2009; Pereira *et al.*, 2006).

Figure 2.1: Fecal-oral route transmission of bacteria. Fecal bacteria such as coliform and *E. coli* came from animal reservoirs and human feces.



Adapted from (Jim Walker, 2010)

Toxicgenicity associated with bivalve consumption can be classified to naturally occurring harmful algae, pathogenic bacteria and viruses. Algal bloom that rich with plankton biomass helps in bivalves production due to high densities of nutrient from soil and environment. At the same time, algae consist substances that can be harmful due to accumulation of toxin producing organisms such as diatoms and cyanobacteria (Rehnstam-Holm *et al.*, 2005) . Moreover, foodborne illnesses are vulnerable to several group of people such as children, elderly, pregnant women, people with immunosuppressive disorder (HIV, cancers), chronic illness (diabetes mellitus and kidney problems and steroid dependent patient (Joan B. Rose *et al.*, 2001).

Although bivalves are believed to act as a vehicle for disease transmission, annual production of bivalves increased enormously (FAO, 2014). This is because bivalve is good source of edible protein and suitable to consume through low or minimally processed such as raw or lightly cooked (Murchie *et al.*, 2005). When consume raw, only few additives or flavored will be added on bivalves while tenderness and easily digested bivalve make it one of consumer's demands (Oliveira *et al.*, 2011).

Bivalves can be considered as significant to human diet because it becomes alternative protein source after fish and other seafood. Aquatic animals have been recommended as good source of high biological value protein and source of polyunsaturated fatty acids (Ruxton *et al.*, 2005). In fact, value of protein in bivalve almost equivalent to milk and egg protein value (Murchie *et al.*, 2005; Sapkota *et al.*, 2008). Despite showing several positive roles towards human diet, number of foodborne disease due to bivalves ingestion is an issue that need to ponder. Since most of bivalves are harvested in sea coastal region and most people consumed it raw. Consumers also can get bivalves directly from fishermen or fishing bay vendors. Therefore, the risk to have foodborne disease due to ingestion of bivalve consumption is much higher.

2.2 Occurrence of foodborne outbreaks due to bivalves consumption in worldwide.

Based on epidemiological data, many infectious outbreaks regarding bivalves consumption have been reported since 1970s. From 1969 to 2000, 46% of outbreaks associated with bivalves shellfish from North America (28%), Europe, Australia and New Zealand (13%). Recorded outbreak from Asia mostly occurred in Japan and China, which contribute 13% of whole outbreaks for same period. From all these recorded outbreaks, oysters are the highest bivalves species that lead to outbreaks because oyster are commonly consumed raw and lightly cooked (Potasman *et al.*, 2002).

In developed countries, they normally record and investigate the etiology of outbreaks. Thus, most of reliable data from these countries can be obtained easily. However, different situation happened in most developing countries when foodborne illness occurred but not reported such as in Africa and South East Asia (Potasman *et al.*, 2002). After several studies in seafood associated outbreaks being conducted, 14 cases (9%) attributed by bacteria and 69 cases (47%) due to viruses contamination and 65 (44%) due to chemical agent (Oliveira *et al.*, 2011).

Additionally, data from Food-Borne Disease Outbreak Surveillance System and the Cholera and Other Vibrio Illness Surveillance System (COVIS) from 1973 to 2006, outbreaks related to seafood associated infections were 188 outbreaks in USA and 143 or 76.1% of outbreaks due to bacterial infections (Iwamoto *et al.*, 2010). This finding proved that bacteria are main contributor to the bivalves contamination. In the meantime, studies about bacterial and viral pathogen in oysters in USA showed that vibrio species is the main bacterial agent contributed to the cases, which is encounter 50% of the cases (DePaola *et al.*, 2010).

In Sweden, a huge foodborne outbreak due to consumption of mussels was occurred in late 2002. Mussels that harvested commercially on Swedish west coast had been exported to several countries in Europe: Norway, Denmark, Belgium and Netherlands. Data recorded shown 82 consumers reported having foodborne illness symptoms in Netherlands and 403 reported cases in Belgium. All infected individuals experienced diarrheas, vomiting, abdominal pain and nausea after 12 to 24 hours mussels' consumption. During investigation, fecal coliform used in analysis and mussels from Swedish west coast harvesting area as comparison sample. Result of this analysis suggested foodborne illness occurred due to combination of contamination agents such as diarrheic shellfish toxins (DST), azaspiracids, noroviruses, enteroviruses and *E. coli* (Rehnstam-Holm & Hernroth, 2005).

A study in Spain examined 127 bivalves mollusks samples showed that bivalve are highly associated with microbial contamination. Moreno Roldan *et al.*, (2011) analyzed several bivalves species such as Mediterranean mussel, smooth clam, grooved carpet-shell clam and striped venus clam from retail fish and shellfish market in 2009. The study had found *E. coli* in 25% raw samples and 4% cooked samples while *Staphylococcus aureus* was recorded as 37% of the samples. Besides that, sample that had been taken during summer shown high microbial load as compared to winter sample.

Latest outbreak due to bivalves consumption was occurred in Zhenjiang province in China. The outbreak occurred in May 2011 when infected individuals reported having diarrhea, abdominal pain, and vomiting. These symptoms happened in 57 individuals at first but the number increased up to 220 suspected cases. Investigation of this outbreak was conducted by comparing exposure to suspected seafood items and cooking methods between 61 probable cases and 61 controls. Thus, investigation found outbreak was attributed to the consumption of contaminated mussels. Diarrheic shellfish poisoning

(DSP) had been clarified as cause of this outbreaks due to present of OA and DTX-1 toxin in mussels (Chen *et al.*, 2013).

2.3 Prevalence of bivalves contamination in Malaysia

There are limited studies about bacterial component in bivalves shellfish have been conducted in Malaysia. Most of study analyzed on well-known bivalves such as blood cockles and oysters species. There are lack of information regarding other bivalves species such as Asian clam, carpet clam and green mussels that harvested specifically in Malaysia. In addition, most of cockles are harvested and cultivated in West Coast of Peninsular Malaysia such as Penang, Perak, Selangor and Johor (DOF, 2012).

Study about bacteriological components in cockles has been conducted which involved three major producing areas: Kuala Sepetang in Selangor, Kuala Sungai Jarum Mas in Perak, and Kuala Juru in Penang. Results of this study reported that cockles harvested in Kuala Juru and Kuala Sepetang have been exceeded safety level with standard plate count (5×10^5 CFU/g), fecal coliform (<300 MPN/100g) and *E. coli* counts (<230 MPN/100g). Cockles that cultivated in Sungai Jarum Mas still under control and fall under safety level for the same parameter (Fisal *et al.*, 2007).

Penang is one of the most urbanized city in Peninsular Malaysia. Therefore, urbanization has been considered as contributing factor towards high number of fecal coliform count in Kuala Juru (Campos & Cachola, 2007). A study of microbiological contamination in bivalves and filter feeder species in East Coast of Peninsular Malaysia was done by Musa *et al.* (2008). In this study, total coliform and *Salmonella* spp. are identified in oysters by using most probable number (MPN) method. This study exposed that all oyster samples are contaminated with several pathogenic bacteria such as *E. coli*, *Klebsiella* spp. and *Salmonella* spp.

Similar study which has been conducted in Setiu, Terengganu revealed that oysters samples contaminated with heavy metal such as zinc (Zn) and pathogenic bacteria. *Shewanella putrifaciens* was detected as predominant bacteria in samples followed by Vibrio species, *Enterobacter cloacae*, *E. coli* and *Chromobacterium violaceum* (Najiah *et al*., 2008). All studies above proven that bivalves in Malaysia also highly contaminated with many pathogenic bacteria.

2.4 Factors influence microbial growth in bivalves

Growth of pathogenic bacteria and microorganisms are affected by many environmental factors such as seasonal influence, geographical properties and pollution level. Seasonal influence strongly correlated with variability of temperature and salinity of water while pollution may cause contamination due to presence of heavy metals in water (Rose *et al*., 2001; Vernocchi *et al*., 2007). All these factors affecting may provide favourable condition to bacterial growth and survival rate then contaminate bivalves directly. Several analytical studies have been done to investigate and demonstrated these phenomena. However, these findings are still controversial due to different geographical area and it depends on the physiological properties of bacteria itself.

Study by Wilkes *et al.* (2009) shown that most of pathogenic bacteria abundant during fall season as compared to the other season. This study involved assessment of microbial indicator for surface waters within agricultural site between 2004 and 2006. This study also suggested indicator bacteria densities strongly associated with heavy rainfall and warmer temperature. Heavy rain cause increase river flow and composition which make water and component in the water such as shellfish, fish and bivalve are risky for contamination (Cooley *et al.*, 2007).

Most of data provide information among four season countries. However, occurrence of microbial contamination in tropical countries also commonly identified. Campos *et al.* (2007) reported high contamination of fecal coliform and *E. coli* in bivalves harvested at Alvor Lagoon especially during rainfall and wet season. This occurrence highly influenced by livestock production and domestic waste from farming site. During rainfall season, high flow water in the river enhance the spreading and accumulation of pollutants to the coastal area (Crowther *et al.*, 2002).

Furthermore, study conducted by Wang *et al.* (2014) also justify and proved similar findings. This study is used to investigate relationship of seasonal dynamics and diversity of bacteria in retail oysters. Result of this study had shown highest microbial load in oysters detected in fall season and not in summer. Favourable environmental condition during fall season such as optimum temperature and better salinity might be a major contributing factor towards this phenomenon. Besides that, proliferated bacteria or parasites during summer are remobilize in fall season (Wilkes *et al.*, 2009).

2.5 Coliform and *E. coli* as microbial indicator.

Total coliform and *E. coli* is one of commonly used microbial indicator in order to determine contamination level in certain food. Basically, total coliform and *E. coli* have fulfill the criteria of microbial indicators, thus majorly acted as microbial indicator for water quality (NHMRC, 2003). Presence of microbial indicator is important in microbiological risks assessment. When microbial indicator is detected, it shows availability and possibility of other pathogenic bacteria in the samples. There are several criteria that must be demonstrated by microbial indicator organisms.

Figure 2.2: Criteria of microbial indicators for fecal contamination.

- be easily and rapidly detectable
- be easily distinguishable from other members of the food flora
- Have a history of constant association with the pathogen whose presence it is to indicate
- always be present when pathogen of concern is present
- be a microorganism whose number ideally should correlate with those of the pathogen concern
- possess growth requirements and a growth rate equaling those of the pathogen
- have a die-off rate that at least parallels that of the pathogen and ideally persist slightly longer than the pathogen concern
- be absent from foods that are free of the pathogen except perhaps at certain minimum numbers

Additional criteria for fecal indicators used in food safety:

- Ideally the bacteria selected should demonstrate specificity, occuring only in intestinal environments.
- They should occur in very high numbers in feces so as to be encountered in high dilutions.
- They should permit relatively easy and fully reliable detection even when present in low numbers.

Adapted from (Doyle & Buchanan, 2007)

2.5.1 Total coliform

Total coliform is a group of bacteria that shared same morphological, physiological and physical characteristics. American Public Health Association (APHA) (2005) has defined total coliform as:

Figure 2.3: Definition and characteristics of coliform

All aerobic and facultative anaerobic, non spore forming, Gram-negative, rod shape bacteria that ferment lactose with gas and acid fermentation at 35°C within 48 hours OR All aerobic and numerous facultative anaerobe, Gram-negative, non spore forming, rod shaped bacteria that grow as red colonies with a metallic sheen at 35°C within 24 hours on an Endo-type medium containing lactose.

Additionally, other characteristics of total coliform that need to include is positive enzymatic reaction with b-D-galactosidase. However, definition of total coliform varies from country to country (Sengupta & Saha, 2013).

Another subset of total coliform is fecal coliform. Fecal coliform is an indicator to determine fecal based contamination in food. Generally, fecal coliforms or known as thermotolerant coliforms are made up with similar physiological and morphological characteristics but temperature of detection are different. Fecal coliform is defined as coliform group bacteria that produce acid and gas from lactose at 44.5 ± 0.2^{0}C within 24 ± 2 h (Ashbolt *et al.*, 2001).

Fecal coliform bacteria normally originated from intestine of mammals and it has short life span as compared to other coliform bacteria. Coliform bacteria include genus *Escherichia, Klebsiella, Enterobacter, Citrobacter, Yirsinia, Serratia* and many more (Sengupta & Saha, 2013). Under coliform genera, *E. coli* occupy 94.1 to 96.8% of in human feces and considered as the largest bacterial group under coliform.