MICROBIOLOGICAL QUALITY OF READY- TO- EAT FOOD SAMPLED FROM THE STREET VENDORS OF KUBANG KERIAN, KELANTAN

By

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A dissertation submitted in partial fulfillment of the requirement for the Degree of Bachelor Health Sciences (Honours) (Environmental and Occupational Health)

JUNE 2015

ACKNOWLEDGEMENT

At the outset, I would like to express my sincerest appreciation to the Almighty for the continuous blessings that provided me the strength and perseverance to be able to carry out this research study positively and complete this dissertation.

This research study and dissertation appears to be in this current form because of great support and assistance from several people. I would like to thank all of them.

First, I would like to express deepest gratitude and much appreciation to my supervisor Dr Hasmah bt Abdullah for the continuous support and kind assistance. I would like to extend my sincere thanks to my co-supervisor Dr Noor Izani Noor Jamil for his kind cooperation throughout the research study. My heartfelt thanks to laboratories officers who helped and teach me throughout the completion process of this dissertation.

And finally an honourable thank you to my family, seniors, classmates and lab partners that continuously give me the drive, understandings and supports throughout my study.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOL, ABBREVIATION AND ACRONYM

-	To / until
%	Percentage
/	Over to
=	Equal to
>	More than
°C	Degree Celsius
CFU	Colony Forming Units
E.coli	Escherichia coli
S.aureus	Staphylococcus aureus
USM	Universiti Sains Malaysia
mL	milliliter
g	gram
WHO	World Health Organization
USFDA	United States Food and Drug Administration
L	Liter
RTE	Ready-To-Eat
TPC	Total Plate Count
APC	Aerobic Plate Count
TCC	Total Colony Count
FC	Fecal Coliform
EMB	Eosin methylene blue
IR	Incident rate

LIST OF SYMBOL, ABBREVIATION AND ACRONYM

N.G.A	*Nasi Gulai Ayam*
N.P.A	*Nasi Paprik Ayam*
N.S.A	*Nasi Sambal Ayam*
N.H.P	*Nasi Hujan Panas*
N.D.G	*Nasi Daging Kerutuk*
N.D.G	*Nasi Daging Goreng*
N.K.I	*Nasi Kerabu Ikan*
N.G.I.K	*Nasi Gulai Ikan Kering*
N.G.I	*Nasi Gulai Ikan*
N.D.I	*Nasi Dagang Ikan*
N.L	*Nasi Lemak*
B.N	*Bubur Nasi*

MICROBIOLOGICAL QUALITY OF READY-TO-EAT FOOD SAMPLED FROM THE STREET VENDORS OF KUBANG KERIAN, KELANTAN

ABSTRACT

Over the years, many food-borne diseases have been reported due to consumption of Ready-To-Eat Food (RTE) that has uncertain microbial quality. The present study was carried out to assess the microbiological quality of various food samples which was vended on streets of Kubang Kerian, Kelantan. A Total of thirty five (n=35) samples were collected from the vendor in an aseptic container and immediately transferred to the laboratory for analysis. The results for microbiological analysis were presented as Total Plate Count (CFU/g), Total Coliform Count (CFU/g), Fecal Coliforms Count or thermotolerant coliforms count (CFU/g), and presence or absence of *E.coli.* It was found that 74.3% out of 35 samples were unsatisfactory for Total Plate Count and 25.7% of the samples were satisfactory. For Total Coliforms Count, 80% samples were unsatisfactory and 20% of samples were satisfactory. In addition, for Fecal Coliforms Counts, 65.7% samples showed unsatisfactory result and 34.3% samples showed satisfactory result. Six out of 35 samples showed presence of *E.coli.* This study recommends some preventive measures which the local authorities and food-maker together should follow, and also maintain the standard hygienic procedure to prepare, cook and handle foods. Implementation of such measures, rules and regulations on street food vendors in Kubang Kerian, Kelantan and restaurant are extremely crucial to maintain the hygienic condition as well as to avoid such spreading of harmful organisms through consumption of contaminated foods.

KUALITI MIKROBIOLOGI SAMPEL MAKANAN SEGERA YANG DIJUAL DI TEPI JALAN SEKITAR KAWASAN KUBANG KERIAN, KELANTAN

ABSTRAK

Penyakit bawaan makanan sering dilaporkan dan seringkali dikaitkan dengan pengambilan makanan segera yang dijual di tepi jalanan. Hal ini kerana makanan ini tidak dipantau cara penyediaannya, pembungkusan serta penyimpanannya dan berpotensi untuk mendapat kotaminasi mikrob yang mampu memberi kesan kesihatan kepada pelanggan. Kajian ini telah dijalankan untuk menilai kualiti mikrobiologi pelbagai sampel makanan yang dijual di tepi jalan di sekitar Kubang Kerian, Kota Bharu, Kelantan. Sebanyak tiga puluh lima (n=35) sampel dikumpulkan secara rawak daripada gerai-gerai di tepi jalan dan disimpan di dalam bekas aseptik untuk analisis makmal. Keputusan analisis mikrobiologi dibahagikan kepada Jumlah Kiraan Plat (CFU / g), Kadar Pertumbuhan Koliform (CFU / g), Kadar Pertumbuhan Koliform Bawaan Najis (CFU / g), dan kewujudan *E.coli*. Hasil dapatan kajian menunjukkan bahawa 74.3% daripada 35 sampel mencatatkan kadar tidak memuaskan untuk Jumlah Kiraan Plat manakala untuk Kadar Pertumbuhan Koliform, 80% sampel adalah tidak memuaskan. Di samping itu, kajian ini juga mendapati sebanyak 67% sampel menunjukkan Kadar Pertumbuhan Koliform Bawaan Najis dan enam daripada 35 sampel menunjukkan kehadiran *E.coli*. Kajian ini juga menekankan kepentingan pelaksanaan peraturan-peraturan serta garis panduan yang ditetapkan di dalam Akta Kualiti Makanan 1983 mengenai pengendali makanan untuk memastikan keadaan kebersihan serta mengelakkan penyebaran organisma berbahaya melalui pengambilan makanan tercemar.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Food borne diseases occur as a result of contamination of food *via* chemical or microorganism. In most cases, factors causing food borne diseases are bacteria, such as *Salmonella* or *Escherichia coli* (*E.coli*), or a virus, such as the *norovirus*. Food borne disease is defined as any illness resulting from the consumption of foods or beverages contaminated with one or more of disease producing agents such as bacteria, parasites, viruses, fungi and their products as well as toxic substances not of microbial origin (Centre of Communicable Diseases, 2006). Diseases linked to food are cholera, typhoid fever, hepatitis A, dysentery and food poisoning. Most of food borne diseases only show symptom after certain amount of time and this makes the root cause is harder to spot. For example, symptoms of food poisoning usually begin 1-3 days after eating contaminated food where individuals feeling sick, vomiting, diarrhea and stomach cramps. Some toxins can cause food poisoning within a much shorter time. It is more common happens after eating at picnics, school cafeterias, large social functions, or restaurants.

The contamination of food may occur in any stage of food production, preparation and consumption. The contamination also can happen in environment setting where there is pollution of water, soil, and air. There are many bacteria that can cause food poisoning, such as *Campylobacter enteritis*, *Vibrio cholerae*, *E.coli, enteritis*, *Staphylococcus aureus*, *Salmonella* and *Shigella*. Infectious organisms including various

bacteria, viruses and parasites or their toxins are the most common causes of food poisoning. *Staphylococcus aureus*, *Salmonella*, *Vibrio parahaemolyticus*, *Clostridium perfringens*, and *Bacillus cereus* are the main foodborne diseases in Malaysia (DeWaal & Robert, 2005).

Infectious organisms or their toxins can contaminate food at any point during its processing or production. Contamination can also take place at home if food is incorrectly handled or cooked. Food poisoning symptoms often include nausea, vomiting or diarrhea, which can start just hours after consumption contaminated food. Most often, food poisoning is mild and resolves without treatment. But some cases are severe, requiring hospitalization.

Ready-To-Eat (RTE) street foods play an important role in people's daily food options as well as their regular nutritional requirements are dependent on these foods, as their ever-growing busy schedule take away the opportunity to eat homemade food and the food is easily accessible as it is sold along the streets to the town . Due to expedient availability, these street foods are one of the primary food options for the locals.

The research is conducted to analyze the bacteriological quality of street food sold by the vendors. It is important to follow the control measures by food venders to improve microbial quality of street vended foods. This will help to ensure that the premises adhere to required hygienic level lined by Ministry of Health Malaysia and comply with Food Act (2009) Section 3(1).

1.2 Research Problem Statement

Food poisoning occurs due to bacterial infections brought through consumption of contaminated food. In food poisoning cases, the agent is carried by the food handler's hands that act as a vector and transmitted to the prepared food (Waller, 2004; Worboys, 1900; Germ Theory, n.d). Food poisoning usually occur as a result of unhygienic food handling either during preparing of the raw material, cooking or serving cooked food.

Street food industry plays an important role in meeting the food requirements of urban dwellers as well as feed the population with wide variety of food that is relatively cheap and easily accessible especially in developing countries. This industry is expected to continue into next century where the urban phenomenon will expand and be part of the culture and life style. Food borne illness of microbial origin is a major health problem associated with food safety. Unfortunately, despite of the high demand in this industry, vendors that cater the food are often poorly educated, unlicensed, untrained in food hygiene and they work with unsanitary condition and lack of knowledge about the causes of food borne pathogen. Consumers are aware that street food is prone to food borne diseases but disregard these health hazard (Hanoshiro *et al.*, 2004; Ghosh *et al.*, 2007).

1.3 Research Objectives

General objective

To determine the microbiological qualities of Ready-To-Eat food sold by street vendors in Kubang Kerian Kelantan.

Specific objectives

a. To determine the Total Plate Count, Total Coliform Count and Fecal Coliform Count in Ready-To-Eat food (RTE) samples sold in streets of Kubang Kerian, Kota Bharu Kelantan.

b. To determine the presence of *E.coli* in Ready-To-Eat food (RTE) samples.

1.4 Rational study

This study seeks to determine the microbiological quality of the Ready-To-Eat food sold in the streets of Kubang Kerian, Kelantan. Ready-To-Eat foods were chosen in this study as there are cases regarding the foodborne disease outbreaks due to consumption of the Ready-To-Eat foods. Based on previous study done in Kota Bharu on the hygienic standard of food premises, unsatisfactory coliform counts were found in 47.8% of total food sampled, that include both the staple and traditional snacks sampled (Zaliha & Mohamed., 2011). Kubang Kerian; a new bustling economic hub was chosen due to its location which is about 7 Km from Kota Bharu, Kelantan, Malaysia and housed 5,259 total population. The street chosen were Jalan Sultan Yahya Petra, Jalan Raja Perempuan Zainab 1 and Jalan Guchil Bayam, Kubang Kerian, Kelantan are the main road in Kubang Kerian which the citizen commutes daily. The road is packed with stalls

selling Ready-To-Eat food to cater the needs. Kubang Kerian is about 10-15 minutes' drive from Kota Bharu, the capital city and 15-20 minutes from the airport in Pengkalan Chepa.

Food safety is a global concern. Foodborne illnesses are prevalent in all parts of the world, and the toll in terms of human life and suffering is enormous. According to the World Health Organization (WHO) (1999) contaminated food contributes to 1.5 billion cases of diarrhea in children each year and more than three million premature deaths (WHO, 1999). Food borne illness also contributes to death in developing countries (Rehydration Project, 2004; WHO, 2002; WHO, 2002b). For example, in the United States, the Centers for Disease Control and Prevention (CDC) the estimations of foodborne diseases cause approximately 76 million illnesses annually among the country's 290 million residents, as well as 325,000 hospitalizations, and 5,000 deaths (Mead *et al.*, 1999). Those deaths and illnesses are shared by both developed and developing nations.

In South East Asia, approximately one million children under five years of age die each year from diarrheal diseases after consuming contaminated food and water. The Star on October 2007 reported food poisoning cases in Malaysia has increased 100% in 2007 with 67% of such cases confined to primary and secondary school students (Zulkifle, 2007). Contamination of food items is frequently found in preparation places of food services premise. However, data on foodborne diseases are extremely scarce and improvements are needed to better identify the causes of foodborne diseases. Thus this study will provide data on level of bacterial contamination on food samples. It also can be used as feedback for government on need for surveillance on food safety. In

Malaysia, the main contributing factor to foodborne diseases was identified as insanitary food handling procedures which accounted for more than 50% of the poisoning episodes.

Food handlers play a major role in the prevention of food poisoning during food preparation; hence, food handler training is seen as one of the main strategies to increase food safety practices. Ehiri and Morris (1996) point out that data on risk factors for foodborne diseases imply that most outbreaks result from faulty food handling practices (Bryan, 1998; Evans *et al.*, 1998). A study in the USA by Howes *et al.* in 1996, recommended that improper food handler practices contributed to approximately 97% of foodborne illnesses in food-service establishments and homes. The majority of food handlers in this study admit that they do not always carry out all the food safety practices they know they should be implementing (Clayton *et al.*, 2010).

Food handlers can help to reduce food poisoning by either preventing contamination of foods or by preventing the growth or survival of bacteria. According to Poh *et al.* (2010), 14 behavior are identified that would prevent contamination of foods and four actions which would prevent the growth and survival of bacteria and four actions which could be defined as contributing to both these hygiene principles. The practices outlined by respondents as being important for preventing food poisoning also appeared to be related to identify risk factors from epidemiological data.

1.5 Experimental Design

The samples were collected by simple random sampling. Thirty five samples (n=35) were collected over a period of three months. Then, microbiological analyses were conducted for Total Plate Count (TPC), Total Coliform Count (TCC), Fecal Coliform Count (FCC) and *E.coli*. The result of the microbiological analyses were compared for compliance to the Microbiological Guidelines for Ready-To-Eat Food and Malaysia Food Act (MOH, 1985) where TPC should be below 10 000 colonies counts per 25 grams of sample, TCC below 100 colonies counts per 25 grams of sample, FCC below three colonies count per 25 grams of sample and absence of *E.coli* for Ready-To-Eat foods to be satisfactory and safe for consumption. The experimental design is illustrated in Figure 1.1.

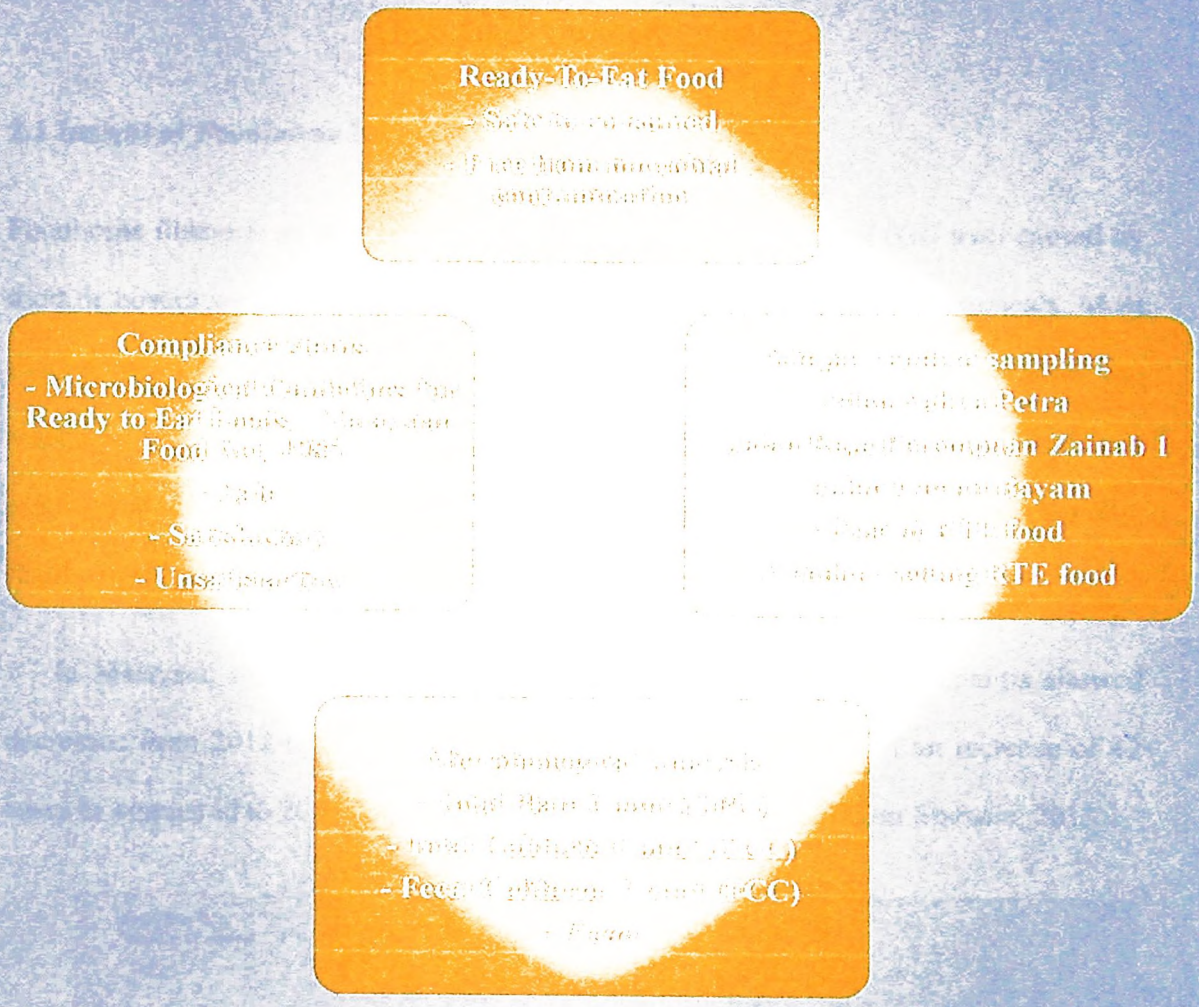


Figure1.1: Research Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

2.1 Insight of Foodborne Illness

Foodborne illness is an infection or irritation of the gastrointestinal (GI) tract caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals. Most foodborne illnesses are acute, meaning they happen suddenly and last a short time, and most people recover on their own without treatment. Common symptoms of foodborne illness include vomiting, diarrhea, abdominal pain, fever, and chills. Occasionally, foodborne illness may lead to more serious complications.

In Malaysia, the number of cases for cholera, typhoid and viral hepatitis showed decreases from 2012 to 2011. On the other hand, dysentery showed an increase of 42 cases as compared to 2011 (Figure 2.1) (Compendium of Environment Statistic, 2013).

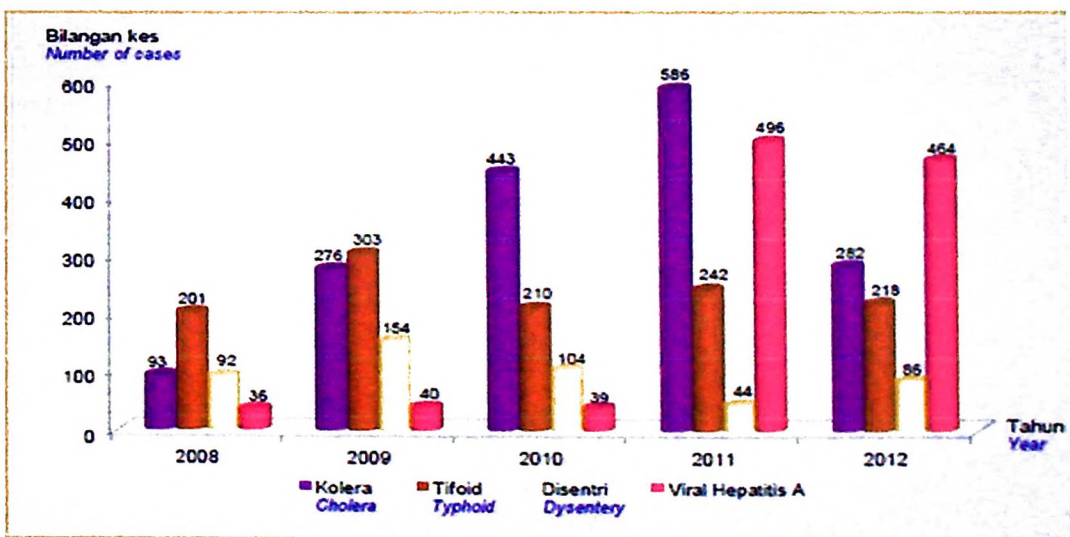
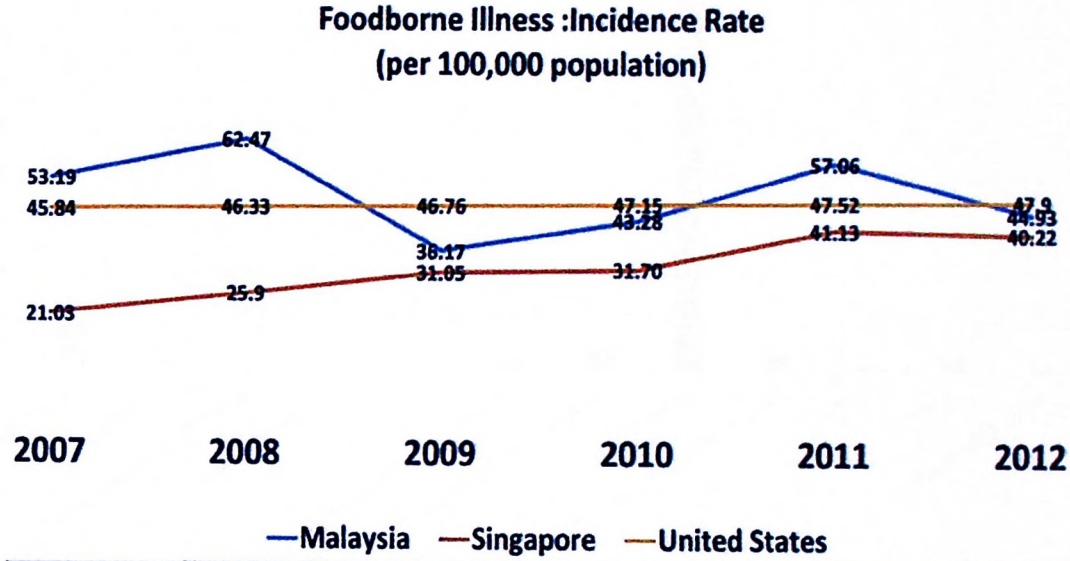


Figure 2.1: Number of Selected Cases of Food and Water Borne Diseases, Malaysia, 2008-2012

Source : Compendium of Environment Statistics 2013

Malaysia shows decreasing number of food poisoning cases in 2012 compared to 2007 which is 53.19 cases per 100,000 populations in 2007 and 44.93 cases in 2012 (Figure 2.2). Despite that Malaysia still has the highest incident rate since 2007 to 2012 compared to Singapore and United States (Aaisah, 2014).



Country/Year	2007	2008	2009	2010	2011	2012
Malaysia	53.19	62.47	36.17	43.28	57.06	44.93
Singapore	21.03	25.9	31.05	31.70	41.13	40.22
United States	45.84	46.33	46.76	47.15	47.52	47.9

Figure 2.2: Comparison of Incident Rate in Malaysia, Singapore and United States
Source : Environmental Health Conference 2014.

Meanwhile, 263 from 1060 sample collected shows contamination by food pathogen where 165 sample positive *E.coli* species and *Salmonella sp.* in 20 cases.

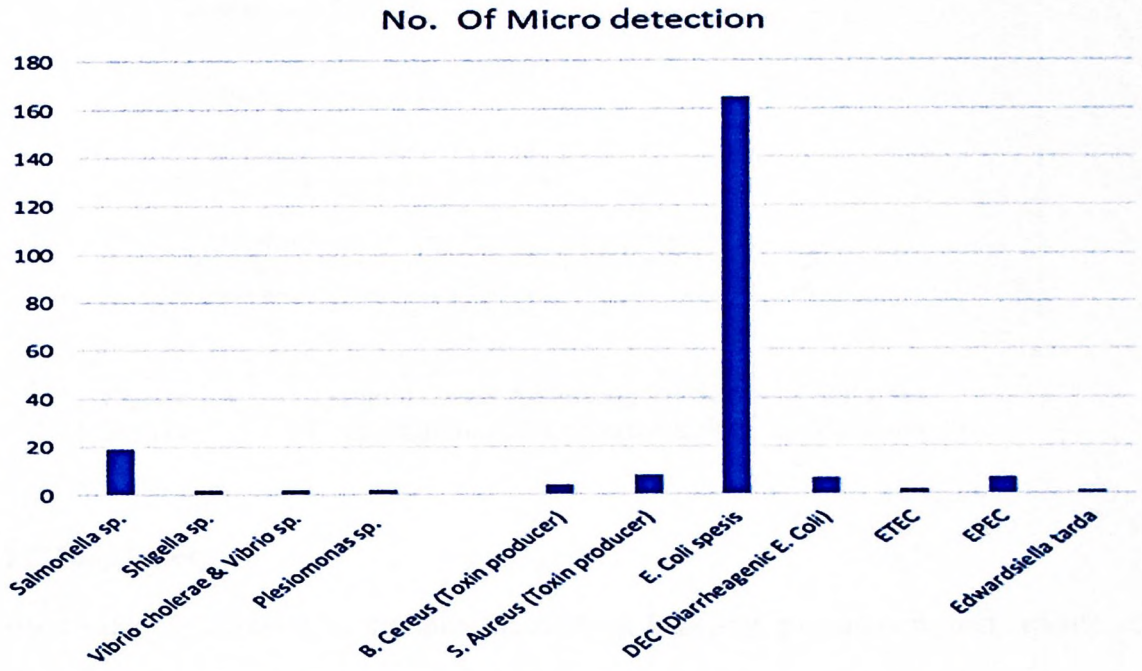


Figure 2.3: Isolated Pathogen 263/1060 (24.8%) Microorganism Detected
Source : Aaisah, 2014. Lab-based surveillance – Acute Diarhoeal Disease Project 2013

Typhoid

Typhoid fever is a bacterial disease triggered by *Salmonella Typhi (S.Typhi)*. It is transmitted through the ingestion of food or drink contaminated by the feces or urine of infected people. Symptoms usually develop 1-3 weeks after contact. In 2012, Kuala Lumpur registered the highest incidence rate (IR) at 2.0 IR, while Federal Territory of Labuan, Perlis and Melaka recorded the lowest IR at 0.0 per 100,000 populations respectively (Figure 2.4). Kelantan shows the second highest incidence rate for typhoid cases which is 1.9 IR per 100,000 populations.

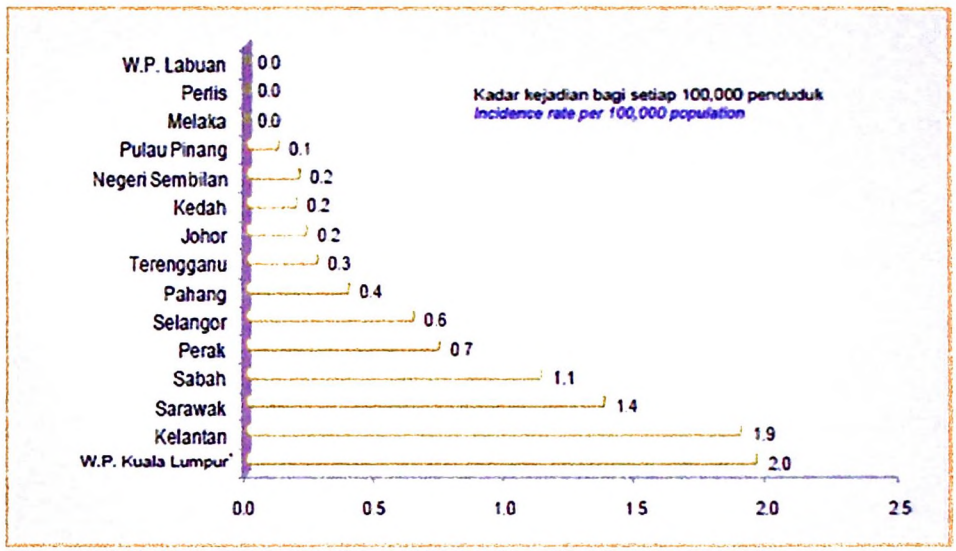


Figure 2.4 : Typhoid Cases According To States in Malaysia
Source : Compendium of Environment Statistic Malaysia 2013

2.2 Food Safety

Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent food borne illness. This includes a number of routines that should be followed to avoid potentially severe health hazards. Food can transmit disease from person to person as well as serve as a growth medium for bacteria that can cause food poisoning (Shiklomanov, 2000). Debates on genetic food safety include the impact of genetically modified food on health of further generations and genetic pollution of environment, which can destroy natural biological diversity (Margaret, 2010). In developed countries there are intricate standards for food preparation, whereas in less developed countries the main issue is simply the availability of adequate safe water, which is usually a critical item.

2.3 Levels of Total Plate Count

The level of TPC or aerobic plate count varies according to classification of the food type and the processing or handling of the food (Food Quality Check Program, 2013; Food Standards Australia New Zealand, 2001). It is divided into three levels which are:

Level 1 - Applies to Ready-To-Eat foods in which all components of the food have been cooked in the manufacturing process/preparation of the final food product and, as such, microbial counts should be low.

Level 2 - Applies to Ready-To-Eat foods which contain some components that have been cooked and then further handled (stored, sliced or mixed) prior to preparation of the final food or where no cooking process has been used.

Level 3 – TPC is not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls). It would be expected that these foods would have an inherent high plate count because of the normal microbial flora present.

Since TPCs shows the count of total count aerobic, mesophilic organisms such as bacteria, yeast and mold fungi on an agar plate at a controlled temperature of 30 °C, it is unsuitable to solely determine microbiological quality of a food based on TPCs alone. The significance of high (unsatisfactory) TPCs cannot truly be made without identifying the microorganisms that predominate or without other microbiological testing (Food Quality Check Program Microbiological Recommendations and Sampling Schedule, 2015; Food Quality Check Program, 2013; Food Standards Australia New Zealand, 2001). Therefore, several indicators are needed to determine the microbiological quality

of the street food. For example *Enterobacteriaceae* which it includes many bacteria that are found in the human or animal intestinal tract, including human pathogens such as *Salmonella* and *Shigella*. *Enterobacteriaceae* are useful indicators of hygiene and of post-processing contamination of heat processed foods (Microbiological quality guide for Ready-To-Eat foods, 2009). Their presence in high numbers in Ready-To-Eat foods indicates that an unacceptable level of contamination has occurred or there has been under processing.

Other than *Enterobacteriaceae*, the presence of *Escherichia coli* also can indicate the microbiological quality of the food. The presence of *E.coli* in Ready-To-Eat foods is undesirable because it indicates poor hygienic conditions which have led to contamination or inadequate heat treatment. Ideally *E.coli* should not be detected and as such a level of <3 per gram (the limit of the Most Probable Number test) has been given as the satisfactory criteria for this organism (Food Quality Check Program, 2013). Levels exceeding 100 per gram are unacceptable and indicate a level of contamination which may have introduced pathogens or that pathogens, if present in the food prior to processing, may have survived.

2.4 Food Safety Issues and Ready-To-Eat Food

Food safety issues is not limited to hygienic food preparation by food handlers, but also applied to Ready-To-Eat (RTE) food. Ready-To-Eat food is defined as food and beverages prepared and sold by vendors on street and other public places for immediate or later consumption, without any heating process needed (Definitions: Food Safety,2015). RTE food which does not need thermal processing before consumption

could be a vehicle for the spread of antibiotic-resistant microorganisms. The food handlers that cater RTE food, they may be lack or no knowledge on how to a diseases transmitted *via* food to their consumer. They also often poorly educated, unlicensed, untrained and sometimes work under crude unsanitary conditions.

Most of the food prepared is not protected from flies which carry food borne pathogen (Essential Safety Requirements For Street-Vended Foods, 1996). Street food rarely stored in a suitable temperature which it aid to promotes bacterial growth and contribute not only to spoilage, but also food poisoning. Therefore, the consumer is exposed to health effects caused by contamination of food by *E.coli*, *Salmonella typhi*, *Pseudomonas* species, *Staphylococus aureus* and *Proteus* species during the food preparation (Hocking, 2003).

2.5 Typical Pathogen Found In Food

The majority of foodborne illness is caused by certain strains of bacteria and viruses. Parasites and chemicals may also cause foodborne illness. For example, *Botulish, Rotavirus, Campylobacter, Salmonella, Crypto, Shiga-toxin producing E coli (STEC), Shigella, Cylospora, Eschericha coli, Staphylococcus aureus, Giarda, Toxoplasmosis, Listeria, Norovirus* and other pathogens (Centers for Disease Control and Prevention, 2014).

The world of foodborne microbes contains a mix of approximately 250 different types of bacteria, viruses, parasites, molds, and algae that are known to cause disease in humans and are therefore called foodborne pathogens. What they all have in common is that they are most often too small to be seen without a microscope, they have simpler

structures and functions than higher plants and animals, and they are able to be cultured in laboratory settings with prescribed methods that aid in their identification (STOP Foodborne Illnesses, 2015).

The term foodborne pathogen loosely describes the microbes that are found in animals (in farm/zoo animals and pets) and in the environment (soil, water and air) that make people sick regardless of how they became infected. Usually, infection happens by direct ingestion of a contaminated product, but it can also happen by contact with other individuals or contact with an animal or pet (Pathogen 101, 2015). Some foodborne microbes make people ill by forming toxins in foods that affect the gut or the neurological system. When an illness is caused by a ingesting a toxin and causes an intoxication it will generally make people sick faster than other foodborne pathogens which cause an infection.

2.5.1 Bacteria

Bacteria are the largest group of problematic foodborne pathogens by far. They are small, one-celled microbes that come in many shapes and are capable of reproducing themselves. Typical cell shapes include spherical (cocci), rod-shaped (bacilli), and curved or comma-shaped (spirillar). These shapes can be seen under the microscope when stained in the laboratory with a Gram stain or dye. Whether or not bacterial cells stain Gram-positive (retaining a crystal violet color) or Gram-negative (those losing the color) also aids in identifying what bacteria are present and what treatments to administer. An important substructure of bacteria is the flagella, a hair-like tail that is responsible for bacterial movement and used to classified and identified on the basis of

their flagella (Pathogen 101, 2015). Much of modern foodborne microbiology is devoted to keeping pathogenic bacteria out of food products and preventing their growth if they are present. *Salmonella*, *E.coli*, *Listeria*, and *Shigella* are well known species of foodborne bacteria.

Harmful bacteria may already be present in foods when they are purchased. Raw foods including meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products, and fresh produce often contain bacteria that cause foodborne illness. This contamination can occur at any time during growth, harvesting or slaughter, processing, storage, and shipping of food (Centers for Disease Control and Prevention, 2014).

When food is stored incorrectly, it gives bacteria the opportunity to multiply; room temperature (between 40 and 140F) is an ideal condition for bacteria to grow. Refrigerated food items should be kept at 40F or below, while hot foods should be kept at 140F or above. At -18°C, there is no bacteria growth detected but the growth spiked at 1 °C -4 °C and at 20 °C - 50 °C, the growth double within 10 or 20 minutes.

Refrigeration can prevent bacteria from growing, and freezing food can further slow or even stop this growth (Cause and Prevention Of Foodborne Illness, 2015). However, bacteria in refrigerated or frozen foods become active again when food is brought to room temperature, so these foods should be thoroughly cooked to kill any harmful bacteria (Ways to Avoid and Reduced Foodborne Illness, n.d).

According to the 2005 FDA Food Code, the danger zone is defined as 41°F - 135°F (5°C - 57°C). Potentially hazardous food should not be stored at temperatures in this range in order to prevent food borne illness, and food that remains in this zone for

more than four hours must be discarded (Centers for Disease Control and Prevention, 2010).

Escherichia coli (E.coli)

Figure 2.5: *Escherichia coli (E.coli)*
Source: Pathogen101:*E.coli* from http:// www.stopfoodbormeillness.org

Escherichia coli (abbreviated as *E.coli*) are a large and diverse group of Gram-negative, bacilli bacteria that normally live in the intestines of people and animals. Most *E.coli* is harmless and actually is an important part of a healthy human intestinal tract (E.coli, 2012). However, some *E.coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract (*Escherichia coli*, 2014). Pathogenic *E.coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic *E.coli*. The types of *E.coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons (Causes of Food Poisoning, 2015).

Some kinds of *E.coli* cause disease by making a toxin called Shiga toxin. The bacteria that make these toxins are called "Shiga toxin-producing" *E.coli* or STEC for short. You might hear these bacteria called verocytotoxic *E.coli* (VTEC) or enterohemorrhagic *E.coli* (EHEC); these all refer generally to the same group of bacteria (E.coli Infections, 2015). The most commonly identified STEC in North America is *E.coli* O157:H7 (often shortened to E.coli O157 or even just "O157").

2.5.2 Viruses

Viruses also cause food borne diseases. Viruses are tiny capsules, much smaller than bacteria that contain genetic material. Viruses cause infections that can lead to illnesses and can transmit the viruses to others (Howard *et al.*, 2014). People who are infected with a virus may contaminate food and drinks, especially if they do not wash their hands thoroughly after using the bathroom. In some cases, the person preparing the food ends up allowing his or her own fecal matter into the food especially if the person has not washed his or her hands thoroughly (Major Foodborne Pathogens, Parasites, Microorganism, Viruses and Natural Toxin, 2015). The common sources of foodborne viruses include food prepared by a person infected with a virus, shellfish from contaminated water and produce irrigated with contaminated water.

2.5.3 Parasites

A parasite is a type of germ (or microbe) which lives on or within any living thing, for example human beings. They are often found in the digestive systems of many animals, for example cats, pigs, cattle but are also found in water based environments such as lakes, ponds and rivers (Parasites- A Guide To Food Poisoning, 2015). These parasites

are single cell organisms or a type of tapeworm which consume blood or other nutrients within the small intestine, resulting in an inflammation within that area.

Cryptosporidium parvum and *Giardia intestinalis* are parasites that are spread through water contaminated with the stools of people or animals that are infected. Foods that come into contact with contaminated water during growth or preparation can become contaminated with these parasites (Scallan *et al.*, 2011). Food preparers who are infected with these parasites can also contaminate foods if they do not thoroughly wash their hands after using the bathroom and before handling food.

2.5.4 Chemicals

Harmful chemicals that cause illness may contaminate foods such as fish or shellfish, which may feed on algae that produce toxins, leading to high concentrations of toxins in their bodies. Some types of fish may be contaminated with bacteria that produce toxins if the fish are not properly refrigerated before they are cooked or served (Michelle *et al.*, 2013). Certain types of wild mushrooms have its natural toxic content that may pose health hazard to human. The usage of excessive pesticides may cause residue on unwashed fruits and vegetables for consumption.

2.6 Bacteria in RTE Food and Antibiotic Resistant

As part of general microbiological safety checks, staphylococci are routinely enumerated in these kinds of foods. However, the presence of antibiotic-resistant staphylococci in RTE food is not routinely investigated, and data are only available from a small number of studies. The present study evaluated the pheno- and genotypical antimicrobial

resistance profile of *Staphylococcus spp.* isolated from 858 RTE foods (cheeses, cured meats, sausages, smoked fishes, salads).

Table 2.1: Changes Pattern of Food-Borne Illness

Traditional	New & Emerging
Usually local	Widespread, multi-state, international
High dose	Low infective dose
Organism readily isolated	Risk undetected by normal method
Normal food processing will eliminate pathogen	High may survive some process
Low mortality/ easily treated with antibiotic	Mortality/ resistant to many antibiotic
Traditional food know to be associated	Food not previously associated with food-borne illness

Source: Ahmad, 2013 Food Safety Malaysia

Of 113 strains isolated, *S. aureus* was the most prevalent species, followed by *S. xylosus, S. saprophyticus,* and *S. epidermidis*. More than half (54.9%) of the isolates were resistant to at least one class of tested antibiotic; of these, 35.4% of the strains were classified as multidrug resistant. Most of the isolates were resistant to cefoxitin (49.6%), followed by clindamycin (39.3%), tigecycline (27.4%), quinupristin-dalfopristin (22.2%), rifampin (20.5%), tetracycline (17.9%), and erythromycin (8.5%).

All methicillin-resistant staphylococci harbored the mecAgene. Among the isolates resistant to at least one antibiotic, 38 harbored tetracycline resistance determinant tet (M), 24 harbored tet (L), and 9 harbored tet (K) (Wioleta, 2013). The results indicated that retail RTE food could be considered an important route for the transmission of antibiotic-resistant bacteria harboring multiple antibiotic resistance genes (Chajęcka & Wioleta, 2014).

Another study conducted by Sharma and Mazumdar (2014) where from total of 37 street vended food samples collected in Streets of Silchar City, Assam India and the isolates were identified as *Escherichia coli* (37.5%), *Pseudimonas aeruginosa* (3.57%), *Staphylococcus aureus* (14.20%), *Salmonella sp.* (5.36%), *Klebsiella sp.* (10.71%), *Shigella sp* (19.64%) and *Enterobacter sp.* (8.93%) respectively. All the 56 isolates were susceptible to ciprofloxacin while their susceptibility to the other drugs varied (Sharma & Mazumdar, 2014).

CHAPTER 3

METHODOLOGY

3.1 Study Location

This study was conducted in Kubang Kerian, Kota Bharu, Kelantan. Kubang Kerian is a new bustling town that is close to Kota Bharu. All the permanent food outlets numbering 1 to 23 located within the 2 Km radius of HUSM, a total of 7 food outlets were randomly chosen (Appendix A).

3.2 Scope of Study

The study seeks to determine Total Plate Count, Total Coliforms Counts, Fecal Coliforms Counts, and presence of *E.coli* in RTE food from the streets of Kubang Kerian in Kota Bharu, Kelantan. To ensure proper assessments were conducted, proper counter measures were taken to ensure there is no contamination throughout the sampling process.

3.3 Sampling Method

The food samples are collected and analyzed for aerobic plate count, total coliform, fecal coliform and presence of *E.coli*. The food samples purchased randomly from various street-food vendors collected aseptically in sterilized containers and was maintained in chilled state using coolants (Johnson,n.d). The pH of the samples was measured using a digital pH meter and then the samples were processed for further studies. The samplings were conducted from 7.30 am until 12.00 afternoons which is the business hour for the stalls.

3.4 Study Period

The study was conducted for 3 month starting from December 2014 until February 2015. The data collections were conducted simultaneously with sampling and laboratory analysis. The collected samples should not be retained more than 2 hour and always kept covered in an ice box.

3.5 Sample Collected

In this study, various types of RTE foods such *Nasi Gulai Ayam* (N.G.A), *Nasi Paprik Ayam* (N.P.A), *Nasi Sambal Ayam* (N.S.A), *Nasi Hujan Panas* (N.H.P), *Nasi Daging Kerutuk* (N.D.G), *Nasi Daging Goreng* (N.D.G), *Nasi Kerabu Ikan* (N.K.I), *Nasi Gulai Ikan Kering* (N.G.I.K), *Nasi Gulai Ikan* (N.G.I), *Nasi Dagang Ikan* (N.D.I), *Nasi Lemak* (N.L), *Pulut* and *Bubur Nasi* (B.N) were sampled along the streets of Jalan Sultan Yahya Petra, Jalan Raja Perempuan Zainab 1 and Jalan Guchil Bayam, Kubang Kerian, Kota Bahru, Kelantan. 35 food samples are collected randomly from 7 stores representing 30% of the total street vendors found in Kubang Kerian, Kelantan by simple ballot. The food chosen pre-packaged food sold in stalls by the street of Kubang Kerian, Kelantan.

3.6 Inclusion Criteria:

- Food sold at street of Kubang Kerian , Kelantan
- Food chosen is ready to eat or pre-packaged food and food sold in categorized premises
- The store is uncategorized, unlicensed or both.
- Have permanents structure/ stall.