# Microbial Safety of Street Food (Fish Satay) in Jeli, Kelantan

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DECLARATION

I would like to declare, this thesis work almost all my own, with explicit and recognised contributions from others. All supporting materials and resources have been properly referenced.

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## ABSTRACT

This study was about microbial assessment towards street food in Jeli, Kelantan, specifically for fish satay. Street food was popular because it has become an alternative food to home cooking and was more economical than restaurants. However, street food was criticized for being processed and sold in unhygienic and open areas. The method used in this study was Standard Plate Count (SPC). SPC used to estimate the population of bacteria in fish satay. Tenfold duplicate serial dilution was prepared from $10^{-1}$ to $10^{-6}$ to enumerate bacteria in a food sample. Selective media were used in this study to identify different types of bacteria: salmonella shigella agar, eosin methylene blue agar, and Bacillus cereus agar. Results showed that all the food samples fish satay FS1, FS2, and FS3 were contaminated with various bacteria that grow under aerobic conditions. The total plate count in sample FS1 is 2.5×107 CFU/ml less than sample FS2 ($13.0\times10^{7}$ CFU/ml) and sample FS3 (tntc). The total bacterial count of Salmonella Shigella agar in sample FS1 was not detected but detected in sample FS2 ($6.0\times10^{4}$ CFU/ml) and sample FS3 (tntc). The total bacterial count of Eosin Methylene Blue agar in sample FS1 was not detected but detected in sample FS2 ($1.62\times10^{5}$ CFU/ml) and sample FS3 (tntc). Finally, the total bacterial count for Bacillus cereus agar in sample FS1 was $3.50\times10^{5}$ CFU/ml but, in samples FS2 and FS3, it was tntc (> 300). In conclusion, the food sold on the streets of Jeli, Kelantan, was contaminated with bacteria. Since it does not meet the general criterion for microbiological guidelines of raw street food, the tabulated result indicates that the fish satay is not safe to consume on its own. Numerous germs on the food sample fish satay can cause foodborne diseases, and the consumption of contaminated street food could risk public health.

Keywords: microbial safety, food bacteria, fish satay, street food, food safety

# ABSTRAK

Kajian ini adalah mengenai penilaian mikrob terhadap makanan jalanan di Jeli, Kelantan, khusus untuk sate ikan. Makanan jalanan popular kerana ia telah menjadi makanan alternatif kepada masakan rumah dan lebih menjimatkan daripada restoran. Bagaimanapun, makanan jalanan dikritik kerana diproses dan dijual di kawasan yang tidak bersih dan terbuka. Kaedah yang digunakan dalam kajian ini ialah Standard Plate Count (SPC). SPC digunakan untuk menganggar populasi bakteria dalam sate ikan. Pencairan bersiri pendua sepuluh kali ganda telah disediakan dari 10-1 hingga 10-6 untuk menghitung bakteria dalam sampel makanan. Media selektif digunakan dalam kajian ini untuk mengenal pasti jenis bakteria yang berbeza: agar salmonella shigella, agar-agar biru metilena eosin, dan agar-agar Bacillus cereus. Keputusan menunjukkan bahawa semua sampel makanan sate ikan FS1, FS2, dan FS3 telah tercemar dengan pelbagai bakteria yang tumbuh dalam keadaan aerobik. Jumlah kiraan plat dalam sampel FS1 ialah 2.5×107 CFU/ml kurang daripada sampel FS2 (13.0×107 CFU/ml) dan sampel FS3 (tntc). Jumlah kiraan bakteria Salmonella Shigella agar dalam sampel FS1 tidak dikesan tetapi dikesan dalam sampel FS2 (6.0×104 CFU/ml) dan sampel FS3 (tntc). Jumlah kiraan bakteria agar Eosin Methylene Blue dalam sampel FS1 tidak dikesan tetapi dikesan dalam sampel FS2 (1.62×105 CFU/ml) dan sampel FS3 (tntc). Akhir sekali, jumlah kiraan bakteria untuk Bacillus cereus agar dalam sampel FS1 ialah 3.50×105 CFU/ml tetapi, dalam sampel FS2 dan FS3, ia adalah tntc (> 300). Kesimpulannya, makanan yang dijual di jalan-jalan Jeli, Kelantan, telah dicemari bakteria. Memandangkan ia tidak memenuhi kriteria umum untuk garis panduan mikrobiologi makanan jalanan mentah, hasil jadual menunjukkan bahawa sate ikan tidak selamat untuk dimakan sendiri. Banyak bakteria pada sampel makanan sate ikan boleh menyebabkan penyakit bawaan makanan, dan pengambilan makanan jalanan yang tercemar boleh membahayakan kesihatan orang ramai.

Kata kunci: keselamatan mikrob, bakteria makanan, sate ikan, makanan jalanan, keselamatan makanan

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# LIST OF SYMBOLS

| | |
|---|---|
| % | Percentage |
| ˚C | Degree Celsius |
| ± | Plus-minus |
| > | Larger than |
| < | Smaller than |
| ≥ | Greater or equal to |
| × | Multiply |

# LIST OF ABBREVIATION

| | |
|---|---|
| RTE | Ready-to-eat |
| FAO | Food and Agriculture Organization |
| WHO | World Health Organization |
| FDA | Food and Drug Administration |
| CFU | Colony Form Unit |
| MSG | Monosodium Glutamate |
| SSA | Salmonella Shigella Agar |
| EMBA | Eosin Methylene Blue Agar |
| BCA | Bacillus Cereus Agar |
| NA | Nutrient Agar |
| SPC | Standard Plate Count |
| TPC | Total Plate Count |
| Ml | Millilitre |
| L | Litre |
| g | Gram |
| SD | Standard Deviation |
| n.d. | Not detected |
| FS1 | Fish satay 1 |
| FS2 | Fish satay 2 |
| FS3 | Fish satay 3 |
| CDC | Centre of Diseases and Control Prevention |

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

This study is about microbial assessment towards street food in Jeli, Kelantan, specifically for the fish-based product, fish satay. The street food (fish satay) can be found in the Jeli area at small stalls, vendors, or night markets which is very popular among local people and travellers. Street food can be defined as cooked food sold for direct consumption by a stall or vendor in a street a public area. Nowadays, street food is trendy because these foods act as an alternative food to home cooking and are more economical than food in restaurants. Because street food is cheaper and widely available, it has become a staple of the local diet. As a result, many individuals rely on street food because there are busy with work and dot have time for cooking (Hossen, et al., 2021).

However, there is an issue regarding street food that is unsafe and not clean because the food is sold by the roadside exposed to dirt and bacterial contamination. The safety of the food sold on the streets is still in doubt, and street food vendors pose a risk of food illness (Jores, Arif, & Rahman, 2018). Street food can be harmful to humans, leading to food poisoning or foodborne illness. Street food has been criticized for its location in which food is processed and sold in an unhygienic and open area (Sezgin & Sanlier, 2016). According to the previous study, microbial contamination in street food will cause foodborne illness and food poisoning due to toxin production from a microbe or improper handling of the food during processing (Khairuzzaman, Chowdhury, Zaman, Al Mamun, & Latiful Bari, 2014).

Food safety issues significantly threaten the safety of consumers in developed countries. Street food is essential in delivering inexpensive, healthy, and affordable food to many urban residents, but it can increase food safety issues when made in the unhygienic and non-regulated climate. Although customers are aware of street food contamination by microorganisms, sellers are not aware of hygiene, and raw or not well cooked, they choose it because the food is cheaper, tasty, and fast service. The government should educate both sellers and consumers about the hygiene and sanitation of street food. This is because education can help minimize foodborne diseases. According to World Health Organization (1996), food sold on the streets can be dangerous to the public's health. This is because the street food lacks essential services and infrastructure, such as adequate water supply and the food handlers lack food safety knowledge.

## 1.2 Problem Statement

The poor environment will lead to foodborne disease caused by pathogenic and non-pathogenic microorganisms. Hassan and Fweja (2020) discovered that the cleanliness of the food stall's environment is essential for guaranteeing food safety throughout the preparation, serving, and even storage of the food. The location of street food nearby disposal areas and the busy road can cause contamination of the street food. Several factors contribute to the risk of eating street food located by the roadside, such as the prevalence of insects, rats, domestic animals, and air pollution (Mjoka & Selepe, 2017).

Food hygiene practices are still lacking among street food workers. A previous study shows that half of the sellers (50%) had no regular handwashing with soap and water (Amarae, et al., 2019). Vendors must be aware of food hygiene and safety during the entire business process, from raw materials until served food to the customers. Typical bacteria are due to improper handling practices among street food workers and causing food poisoning. According to Rane (2011), the leading cause of bacterial contamination includes preparation sites, cooking utensils and serving equipment, raw materials, the abuse of cooked foods by time/temperature and seller personal hygiene.

Raw processed food is easily contaminated when not stored at the correct temperature. Fish satay is a frozen processed food that must be kept at a low temperature -18˚C. However, food handlers did not provide appropriate storage to store the fish satay at the stall, leading to the growth of bacteria. Street food that are contaminated with bacteria results in food poisonings such as diarrhea, vomiting, stomach pains, and nausea ( Marutha & Chelule, 2020).

## 1.3 Hypothesis

$H_0$: The total bacteria count in the street food sample exceed the limit Microbiological Guideline

$H_1$: The total bacteria count in the street food sample did not exceed the limit Microbiological Guideline

## 1.4 Scope of Study

The general purpose of this study is to determine bacteria contain in street food (fish satay) for the safety and quality of the food product. The research method used is the standard plate count method that can evaluate the level of bacteria-contaminated in the street food product (fish satay). The technique used to grow and identify the microorganism is the culturing method, which inoculates, incubate, isolates, inspection, and identification from the food sample (fish satay). The pour plate method is used in the inoculation stage with complex (non-synthetic) media.

## 1.5 Significance Study

The finding of this study will benefit society, especially consumers who like to eat street food. Consumers must be aware of buying street food in terms of safety and hygiene to prevent food poisoning. Street foods are exposed to many contaminations; thus, this research will help them understand the importance of good hygiene practices in food processing. This research will also benefit students who learn about microbial in food processing. Fish satay is one of the processed foods made from fish, and it is sold at the stall or vendor by the street. Therefore, students will know the type of bacteria contained in processed food. Then, this study will also explain poor environmental conditions that will lead to foodborne illness. Street food is exposed to open areas, unlike processed food sold in restaurants or hotels in closed and clean environments. Food poisoning or foodborne illness are caused by bacteria that contaminate the food in any condition, and this study will discuss the type of bacteria found in fish satay, processed street food in Jeli, Kelantan.

## 1.6 Limitation of study

Some restrictions apply to this study. In this analysis, there might be potential limitations: the lack of previous research studies on the topic of fish satay street food. There is also no case study about fish satay in Malaysia or Thailand. The information and preliminary analysis of fish satay are limited; thus, it is hard to find any case study and research paper about that street food.

## 1.7 Research objective

1. To determine the presence of pathogen bacteria (*Salmonella spp., Bacillus cereus, and Escherichia coli*) in street food fish satay.
2. To determine the microbial safety of street food fish satay and whether it comply with Microbiological Guidelines.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Street food

Street food is defined as ready-to-eat (RTE) food prepared and sold by the roadside, vendor, and hawker (FAO, 2009) or other public places such as local markets, fairs, or any food event. Street food is for rapid consumption where most of the food is obtained from street vendors, as they use portable food booths, food carts, and mobile stalls like food trucks and carts. Depending on the weather, stalls for vendors are generally positioned outside, under a roof, easily accessible from the street. These places sometimes provide low-cost seating space that is occasionally simple in design or does not have any facilities at all where people will buy the street food and eat them instantly while standing or walking. Their marketing success is solely dependent on their area and word-of-mouth promotional strategies. However, nowadays, we can see much advertising of street food using an online platform—the management and operation of street food are usually responsible by individual or family businesses.

Street food can be found mainly in an urban area where it has become one of the significant food consumptions daily (Sualeh & Zakir , 2020). It has become more common for people to have regular meals from street food as it is convenient and popular among middle-income and low-income communities. According to Solomons (2013) from Encyclopaedia of Human Nutrition, adult employees and high-school students often eat one-quarter or more of their daily calorie intake from street food. However, most street food schoolchildren consume is highly sweetened and usually deep-fried that are not suitable for their growth. This can be understood as expensive living in an urban area than in a rural area. Street foods are cheaper than home-cooked meals, particularly when considering the time spent purchasing and preparing and the cost of transportation and gasoline spent on grocery shopping (Sharma, 2016). Street food has become popular in urban as more women begin to work more individuals work far from home, and poor urban communities lack access to housing and cooking facilities, as groceries are more expensive and harder to get.

Street food is different from a restaurant in terms of serving, customer service, and type of food sold. Street food could be considered snack food before actual mealtime because some street food is small in portion, simple, and a light meal. Meanwhile, food in a restaurant consists of an entire meal course, like a home-cooked meal. Unlike restaurant food, street food undergoes minimum processing, but some are highly processed. People can choose either to eat at the stall or takeaway. Street food is a budget-friendly option that is also delicious. Most street vendors are generally experts at one kind of food, making their products very popular. They grow better at creating local products compared to a restaurant. Besides, nowadays, people are too busy to cook at home because of their hectic schedules that depend on street food. People eat street food for various reasons, including convenience and low cost, as well as the opportunity to try

different ethnic cuisines and support local small businesses and sellers (Sualeh & Zakir , 2020).

Street food is different in other countries and places because street food is eaten primarily for culture and the environment in a specific region. Cultural, racial, and religious differences affect the diversity and quality of such street food. These street foods are primarily prepared and eaten according to tradition and local knowledge (Fellows & Hilmi, 2011). Street food is an essential component of any country's cuisine. These foods and drinks are necessary to spread the local eating patterns worldwide. In addition, they play a vital role in preserving a cultural and social legacy by selling local food at street stands. Tourists who want to sample local cuisine will also find street food appealing. These meals are in high demand due to their taste, ease of availability, low cost, social and cultural connection, and nutritional value (Sezgin & Şanlıer, 2016).

## 2.2 Food contamination

According to Hussain (2016), the contamination of foods is generally described as spoiled or contaminated by microorganisms, such as bacteria or parasites, or by toxic substances that render it inadequate for use. Then, food contamination can be categorized by the type of contamination such as chemical, physical, biological contamination, and the combination is cross-contamination (Australian Institute of Food Safety, 2016). A serious issue that many people worldwide are concerned about is food contamination. Even though improvement and precaution measures are used to control the

contamination, it still occurs in a restaurant, home, school, and street food. (Kamala & Kumar, 2018).

There are a few types of food contamination related to street food, such as biological, physical, chemical and cross-contamination (Canadian Institute of Food Safety, 2019). Biological contamination means that the food is contaminated by any microorganism, insect, pest, or human. Usually, bacteria and viruses are the two most common causes of food poisoning from biological contamination: *Salmonella spp*., *Escherichia coli*, *listeria*, and *Norovirus*. Next, physical contamination could occur when foreign matter contaminates the food during processing at all stages. For example, there is screw in canned food, hair in bread, and plastic in the soup that could injure a person.

Then, chemical contamination means the food is exposed to natural or artificial chemical materials. The toxic chemical is hazardous to humans if it contaminates the food because it can cause death. Finally, cross-contamination combines biological, chemical, and physical contamination in food products. This is the cause of foodborne diseases unsafe for humans and could risk their lives. The possibility for contamination of street meals with dangerous microbes has been widely established, and the eating of infected vended foods has been linked to several outbreaks of illnesses (Imathiu, 2017).

Table 2.1 show bacteria and viruses that are usually contained in street food (Kamala & Kumar, 2018) and (Al- Mamun, Chowdhurry, Biswas, & Absar, 2018)

| Pathogen | |
|---|---|
| Bacteria | *Clostridium botulinum*<br>*Salmonella spp.*<br>*Clostridium perfringens*<br>*Botulinum spp.*<br>*Streptococci spp.*<br>*Lactobacilli spp.*<br>*Shigellas spp.*<br>*Listeria monocytogenes*<br>*Staphylococcus aureus*<br>*E. coli*<br>*Campylobacter jejuni*<br>*Bacillus cereus*<br>*Proteus spp.*<br>*Pseudomonas spp.*<br>*Vibrio spp.*<br>*Brucella spp.*<br>*Clostridium botulinum*<br>*Yersinia enterocolitica* |
| Virus | *Hepatitis A virus*<br>*Rotavirus*<br>*Echovirus*<br>*Calicivirus*<br>*Hepatitis E Virus* (HEV)<br>*Norovirus* (Nov) |

Street food contamination can occur due to several factors influencing the growth of microorganisms, such as improper temperature for cooking and storage, poor hygiene environment, contaminated utensils, and poor employee health. The important in street food processing is the management and handling of raw material until the food is served to the customer to avoid contamination. Besides, a study has shown that poor management at all stages leads to a rapid deterioration in the nutritional value, but it also gives fungal growth and mycotoxin development a proper state (Abdolshahi & Yancheshmeh, 2020). Therefore, street food traders must be aware of surrounding hygiene, and they must practice appropriate food handling to ensure the food served is safe to consume. Besides the stall area, the utensil and other equipment must be clean and sterilized. Any precaution must be considered to avoid cross-contamination that can affect food production.

The previous study reported by Okojie and Isah (2019), in a developing country such as Africa, most street food vendors are not aware of the safety and hygiene of the food production. They also lack food safety training and proper hygiene standards and are not regulated. Street food is at risk for infection or cross-contamination at all processing points because of inadequate personal hygiene of raw materials and the vendor. Another example is a case study from ready-to-eat food in Istanbul, Turkey. The primary factor in microorganism contamination is cross-contamination and infection of food handlers. Cross-contamination occurs when cooked food is placed near raw food and the utensil used for both cooked and raw food (Oz, Karadayi, Cakan, Karadayi, & Cevik, 2014).

## 2.3 Foodborne disease

The eating of infected foods and drinks can cause foodborne diseases. Several forms of foodborne illnesses can cause microbes or pathogens to contaminate foods. Cross-contamination usually results from incorrect food handling, processing, or storage. Foodborne disease can also be transmitted by applying unintended pesticides or antibiotics in food products such as fruits and vegetables as raw foods. Furthermore, the infection of food or the raw material is a source for interaction between food and plagues and between pests such as flies, rodents, and cockroaches. The infections can be caused by bacteria, viruses, and parasites, leading to foodborne illnesses. Some diseases are poisons produced by contaminants or chemicals contaminating finished goods or raw ingredients.

Malaysia has a high incidence of foodborne illness because the country's climate and conditions are ideal for developing most bacteria. As reported by Abdul Mutalib, Syafinaz, Sakai and Shirai (2015), foodborne diseases are common in Malaysia and may be fatal. No matter how terrible the symptoms are, most people who become sick from eating contaminated food will avoid medical care and just go home. Therefore, few cases of food poisoning are documented. The table 2.2 below shows the number of cases and incidence rate of food and water borne diseases in Malaysia from 2009 to 2013 that are recorded by Department of Malaysia in 2014. Food poisoning was the leading cause of food and water borne diseases compared to typhoid, cholera, dysentery, and hepatitis A.

Table 2.2 shows the number of cases and incidence rate of food and water borne diseases in Malaysia, 2009-2013

| Year | Food Poisoning | | Typhoid | | Cholera | | Dysentery | | Hepatitis A | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Case | IR | Case | IR | Case | IR | Case | IR | Case | IR |
| 2009 | 10238 | 36.2 | 303 | 1.1 | 276 | 1.0 | 154 | 0.5 | 40 | 0.1 |
| 2010 | 12519 | 44.2 | 210 | 0.7 | 443 | 1.6 | 104 | 0.4 | 39 | 0.1 |
| 2011 | 16292 | 56.3 | 242 | 0.8 | 586 | 2.0 | 44 | 0.2 | 496 | 1.7 |
| 2012 | 13182 | 44.9 | 219 | 0.8 | 282 | 1.0 | 86 | 0.3 | 464 | 1.6 |
| 2013 | 14202 | 47.8 | 218 | 0.7 | 171 | 0.6 | 83 | 0.3 | 121 | 0.4 |

*IR: incidence rate
Source: Department of Malaysia 2014

The rise in food poisoning cases could imply that food handlers have overlooked the requirement of hygiene practices during handling the food (Abdul-Mutalib, Syafinaz, Sakai, & Shirai, 2015). Besides, Salleh *et al.* stated that the factor that caused foodborne diseases are related to food handlers' attitude and behaviour. Improper handling of the food can contribute to cross contamination in preparation area and leading to foodborne diseases. In the recent years, food poisoning cases are recorded by Ministry of Health in Malaysia in their annual report. The total number of food poisoning cases reported in 2017 is 13, 490 and increase in 2018 which is 14, 732. Then, statistic reported by Hirschmann (2020) the food poisoning cases in Malaysia had increase to 16, 583 cases. The number of cases is increasing in every year could raise health risk to everyone.

Besides Malaysia, foodborne cases occur globally. A previous study showed that about 8,871 foodborne illness cases already happened in Brazil between the years 2007-2016. These infections mainly were because of *Salmonella spp*. Bacteria, followed by *E. coli* and *Staphylococcus spp* (Camino Feltes, Arisseto-Bragotto, & Block, 2017). The outbreak of this disease due to an inadequate processing and subsequent storing of food during manufacture where they did not use proper temperatures is also associated with the high possibility that pathogen bacteria infected the food product.

Figure 2.3 shows foodborne disease data from 2007-2016 in Brazil

| Year | Outbreaks | Ill people | Exposed people | Dead people |
|---|---|---|---|---|
| **2007** | 683 | 11879 | 25195 | 11 |
| **2008** | 641 | 98995 | 23275 | 26 |
| **2009** | 594 | 9431 | 24014 | 12 |
| **2010** | 498 | 8628 | 23954 | 11 |
| **2011** | 795 | 17884 | 52640 | 4 |
| **2012** | 863 | 14670 | 42138 | 10 |
| **2013** | 861 | 17455 | 64340 | 8 |
| **2014** | 886 | 15700 | 124359 | 9 |
| **2015** | 673 | 10676 | 35826 | 17 |
| **2016** | 354 | 5963 | 194724 | 3 |
| **Total** | **6484** | **121283** | **610465** | **111** |

Source: Camino Feltes, Arisseto-Bragotto and Block (2017)

According to Addo-Tham (2020), street food has been attributed to several foodborne diseases and cases of food poisoning. This has contributed to an increasing public health concern about food safety. Improper food handling and waste management are two primary sources that can contaminate the preparation area. It has been reported that 70-90% of cases from animal, insect, and liquid waste causes cross-contamination (Rane, 2011). Before making any food product, food handlers must ensure that their working space is clean and sterile. In addition, personal hygiene practices are essential, especially to food handlers, to avoid any cross-contamination during food processing.

The FDA's CORE Response Team recently investigated a foodborne outbreak in the United States of America. A wide range of outbreaks is investigated, with some in the last phases of research, while others are in the early stages of inquiry. The Centers for Disease Control and Prevention (CDC) provides the FDA with a case count. Case counts are dynamic, and the number of diseases varies regularly over an inquiry. This information is provided to estimate the extent of an outbreak for each week based on the number of cases reported. The table below shows that *Salmonella spp.* and *E. coli* bacteria are the most causes of foodborne illness.

Table 2.4 Show the foodborne illness in United States, reported by FDA

| Date posted | Ref. | Pathogen | Product (s) Linked to illness (if any) | Total Case Count | Investigation Status | Outbreak Status |
|---|---|---|---|---|---|---|
| **11/24/2021** | 1044 | *Salmonella* | Not Yet Identified | 28 | Active | Ongoing |
| **11/17/2021** | 1043 | *E. coli* O157:H7 | Spinach | 18 | Active | Ongoing |
| **9/15/2021** | 1031 | *Salmonella* | Red, Yellow, and White Onions | 892 | Active | Ongoing |
| **9/15/2021** | 1025 | *Salmonella* | Seafood | 102 | Open | Ongoing |
| **7/14/2021** | 1019 | *Salmonella* Typhimurium | Salad Greens | 31 | Closed | Ended |
| **7/14/2021** | 1010 | *E. coli* | Cake Mix | CDC Notice | Closed | Ended |
| **6/9/2021** | 1007 | *Salmonella* | Shrimp | 9 | Closed | Ended |

Source: Food and Drug Administration (2021)

### *2.3.1 Salmonella spp. bacteria*

*Salmonella spp.* is a widespread bacterial condition affecting the intestinal tract of humans. *Salmonella spp.* bacteria usually reside and are expelled by manure in animals and contained in human intestines. People are often infected with *Salmonella spp.* from contaminated water or food. There are no signs of *Salmonella spp.* infection where a person may not have a symptom in most cases. However, some people may have symptoms developed 8 to 72 hours suffer from diarrheal, fever, and stomach cramps. Without medicine, most stable individuals recover within a few days. *Salmonella spp.* can distribute between food processing phases and when people consume raw food or undercooked foods by food handlers or chefs who do not wash their hands or the surfaces and instruments they use. Besides, *Salmonella spp.* can also spread from animals to humans. This is when a person with contact with such animals, including poultry and reptiles, may spread the bacteria from animals to food unless they perform handwashing correctly before working with food. Animals can also transmit the bacteria to homes if they consume *salmonella*-contaminated food.

In 1888, Gaertner's scientists isolated bacteria enteritis from cow meat. Then he also separated the bacteria from a man's organ that ate the cow meat. The first laboratory-confirmed *salmonellosis* outbreak was when the man suffered from food poisoning (Bell & Kyriakides, 2009). The latest cases of *Salmonella* typhimurium outbreak are on January 11, 2021. As reported by CDC, a public health official from multiple states, including Canada, has investigated *Salmonella Typhimurium* infection on hedgehogs. About 49

people have been infected with *Salmonella Typhimurium* in 25 different states. About 11 cases were hospitalized, and no deaths were reported. Then 14 patients were children under five years old. Therefore, people must beware of the animal even it is a home pet. This is because animals can carry ***Salmonella spp.*** in their feces even if they look healthy and clean. These microorganisms can quickly spread to your body, habitat, and all other places in the area where they live. If a person is touching the animals or its environment, after that touching their faces and mouth, then without washing their hands, there is a possibility the person is swallowing the ***Salmonella spp.*** bacteria and getting an infection from it.

Table 2.5 shows diarrheal disease caused by *Salmonella* bacteria

| Year | Salmonella | Sources | Country | Region | No. Of cases |
|---|---|---|---|---|---|
| **2015** | Salmonella Enteritis | Macaroni with cheese and eggs | Bosnia & Herzegovina | | 200 |
| **2016** | Salmonella Enteridis | Cheese pasta | Greece | | 56 |
| **2011-2016** | New Salmonella serotype | Sesame-based products | Europe | Greece, Germany, Czech Republic, Luxembourg | 40 |
| **2017** | | | Europe | | 92,649 |
| **2017** | Salmonella Enteridis | Eggs from Poland | Europe | 8 countries | 196 |
| **2014-2017** | Salmonella Agona | Possibly cucumber | UK, Findland | | 269 |
| **2018-2019** | Salmonella Poona | Infant formula | France, Belgium, Luxembourg | | 32 |
| **2018** | Salmonella group D | Mayonnaise from fast-food vendor | Romania | Lasi | 134 |

Source: National Center for Biotechnology Information, U.S. National Library of Medicine

### *2.3.2 Escherichia coli*

*E. coli* (*Escherichia coli*) is a bacterial species that usually lives in the human gut, and it is also present in some animals' intestines. According to the CDC, most *E. coli* varieties are harmless and safely help maintain the digestive tract. However, if people eat infected food or drink contaminated water, those strains can cause diarrheal. Several strains are responsible for diarrheal, vomiting, stomach pain, and cramping. If not adequately treated, one strain can result in kidney failure. The most common method of getting an *E. coli* infection is to eat infected food. Without treatment, most patients recover after one week. *E. coli* is a genus of rod-shaped Enterobacteriaceae, which can exist with or without air in environments. Then, the symptom could last about 5 to 7 days. Many cases of *E. coli* infections are moderate and do not pose a significant risk for health, and most cases solve themselves, as people can relax and drink lots of fluids. However, such strains can produce severe symptoms and complications that endanger life, such as hemolytic uremic syndrome, resulting in kidney damage and death.

### *2.3.3 Bacillus cereus*

*Bacillus cereus* is a Gram-positive, facultative anaerobic toxin-producing bacteria. Bacteria are common in the environment, soil, and vegetation, which can also be present in food and multiply quickly at room temperature. There are two main types of intestinal diseases caused by *Bacillus cereus*. One is diarrheal, and the other makes nausea or vomiting worse. *Bacillus cereus* is also associated with eye, respiratory tract, and wound infections. The intestinal or non-intestinal pathogenicity of *Bacillus cereus* is closely related to the production of external enzymes that destroy tissues. Among these secreted toxins are four types of hemolysis, three different types of phospholipases, one type of vomiting toxin, and protease.

There are two types of foodborne *Bacillus cereus* disease (McDowell, Sands, & Friedman, 2020). First, contaminated food (due to many types of food left at room temperature) reaches the small intestine, and toxins are large molecules. Thus, this can cause diarrheal, seizures, and sometimes nausea or vomiting. The first type of incubation time is around 6 to 15 hours. The affected food (mainly starchy food, usually rice) contains different toxins for the second type. The toxin causes Bacillus cereus-type vomiting. The incubation time for this type is 30 minutes to 6 hours.

Then, according to Tarek F. and Mansel W. (2013), Food poisoning control should be designed to prevent spore germination and reduce the bacteria's vegetative cell growth. The foods should be cooled quickly to less than seven °C or held above 60°C and reheated thoroughly before serving for food poisoning control. The food must be chilled in the refrigerator to inhibit the growth of any microorganism. As suggested by Food Standards

Australia and New Zealand, people can prepare and serve food properly, then keep it warm (60$^{o}$C – 80$^{o}$C) before eating. If it leaves at room temperature, a high temperature will hinder microbial growth at some periods. If the food is consumed later, cool the food, and it can be packed into a fridge or freezer. Then, a significant proportion of warm food should be divided into smaller containers to cool it and ensure the refrigerator is at the right temperature at 5$^{o}$C or lower. This is to inhibit any microorganism's growth on the food.

*Bacillus cereus* is a foodborne bacterium found in the soil, vegetation, and intestines of insects and mammals. Under harsh conditions, bacteria may become spores that can withstand extreme temperatures and withstand food processing. In addition, spores can accumulate on the surface of equipment used in the food industry, leading to *Bacillus cereus* infection under favorable conditions, for example, in the temperature range of 5°C to 60°C, where the spores can become bacteria and multiply. The previous study reported that *Bacillus cereus* could grow in food at temperatures of approximately 8–5$^{o}$C, but the number of CFU will increase when the temperature is between 30-42$^{0}$C (Jessberger, Dietrich, Granum, & Martlbauer, 2020). *Bacillus cereus* is classified as a species of Psychrotolerant (psychrotrophic) that can develop at cold temperatures. Since then, further experiments have shown growth and development up to 108 cfu/g or ml, and during transport and storage, it can grow at low temperatures between 4–10$^{o}$C.

## 2.4 Street food in Malaysia

In Malaysia, street activity started years ago that can be seen in crowded places like city centres, but nowadays street food can be seen everywhere. The operation can be seen by the roadside, with small stalls selling local food and beverages such as 'cendol,' ice shaved, banana fritters, fish crackers, and more (Sulaiman, et al., 2010). A hawker or street vendor can be identified as a merchant selling goods, water, grocery, or daily products in the streets, lanes, walkways, public parks, or any other public place to the community using temporary shelters or carts. The current study portrayed Malaysia as a foodie's paradise, with a wide variety of local and international cuisines. Street food is popular in Malaysia because it is inexpensive, easy to get, and has various options (Izhab, Dahrial, Bardurdin, & Ying , 2021).

Consumers, particularly in developed countries, are widely accepted in food sold by street vendors. The rapid growth of the food industry has implemented numerous street-selling food choices that then raise customer demand for dining outdoors compared with home-cooked meals. In Malaysia, street-selling drinks and food are well always received by customers due to their tastes, low prices, and frequent availability. A previous study by Latchumaya et al. (2021) stated that the street food concept in Malaysia is popular because it is promoted by street vended and night markets that sell ready-to-eat food. These places are only for a short time, and they are usually in a place where many people live.

According to Dewanti-Hariyadi & Gitapratiwi (2014), Malaysia and other Southeast Asian nations have a high incidence of foodborne illness outbreaks, mainly due to street

food sellers' unclean practices. Street foods are still popular despite the outbreak because street food has become the source of income for local people. In 2012, there were more than 11,000 street vendors in Malaysia, with total revenue of $490 million. Vendors and food handlers in Malaysia make millions of dollars each year from street food selling, and the number of outlets and transactions increase from 6.2% and 5.9% a year between 2008 and 2012 (Shafiee, Ab Karim, Mohamed Razal, & Ungku Zainal Abidin, 2018).

Reported by the Council of Tourism of Terengganu (2019) highlighted many unique street foods found in Terengganu. The commonly highlighted foodsre nasi dagang, nasi kerabu, keropok lekor, satar, bekang, bronok, qasidah, and assam gumpal (Zulkifli, et al., 2020). People can find all this food information on advertising tools, like blogs and tourist websites. It would greatly benefit promoting local food and development, particularly in rural areas. Traditional cuisine and its beauty naturally belong solely to the ethnic identity of a destination such satar originally from Terengganu, but now it can be found in many places in peninsular Malaysia.

### 2.4.1 Street food Keropok Lekor

Fish crackers, or keropok lekor, are a traditional Malay delicacy popular along the Peninsular Malaysian east coast, particularly Terengganu. The term "Lekor" is taken from the Terengganu Malay dialect, which meaning "roll" (Ying & Tengku Anuar, 2019). The coastline of Terengganu is the longest in the Malaysian Peninsula. Because of the

geographical situation, agriculture and fishing were significant economic activities of the locals. The notion of 'Keropok Lekor' is then conceived because of the financial aspect, for most local people serve as fishermen. Different places have another name for keropok lekor. In Kelantan they call it keropok batang and in Pahang they call it as keropok tongkol (Wan Md. Hatta, 2015). Keropok lekor in Terengganu and Kelantan consists of fish as the main ingredients while Pahang uses sago flour as the main ingredients; thus, there is a different taste.

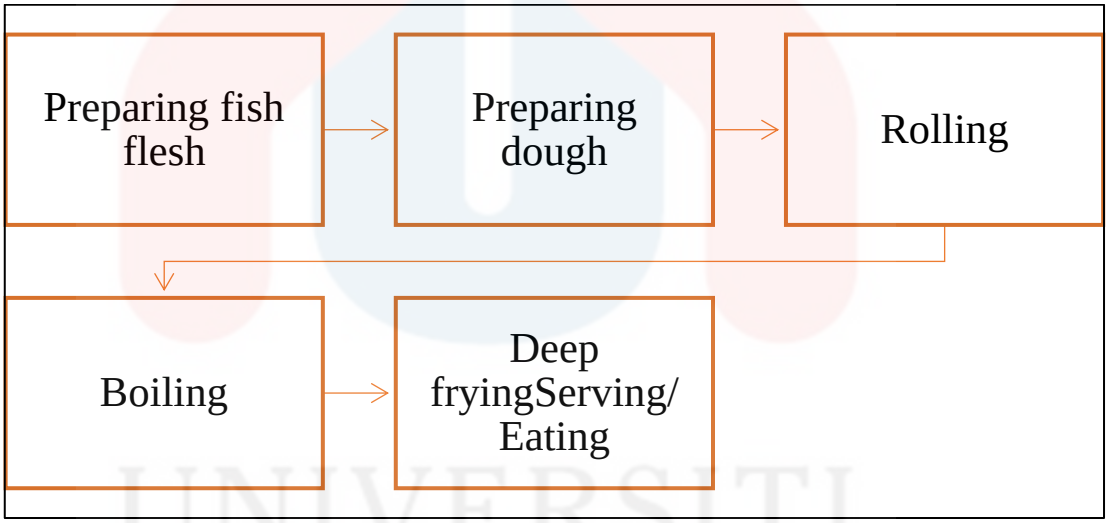



Diagram 2.1 show the process of making keropok lekor in Trengganu (Wan Md. Hatta, 2015)

Keropok lekor has the texture and shape of a chewy sausage that can be eaten either boiled or fried with special sauce. Keropok lekor's main ingredients include freshwater mince fish, water, sago meal or tapioca, salt, glutamate monosodium (MSG),

sugar, and flavoring. Keropok lekor has the shape of a chewy sausage that is either boiled or fried (Lani, Peng , Suhaili, & Hassan, 2017). The processing keropok lekor consists of five phases: mincing, combining the minced fish and other ingredients, kneading, molding the dough, boiling, and cooling before packing. It is available directly at the manufacturing process, hawker stalls, school canteen, and restaurants combined with dipping sauce.

At room temperature, keropok lekor only lasts for a day because water contains in the food can cause microbial growth. The freshly boiled keropok lekor was currently low in microbial numbers, but contamination during the cooling process could occur, and microbiological protection and consistency of this substance could be compromised (Hamat, Lani, Hamzah, Alias, & Hassan, 2019). Therefore, after cooling, there is a possibility microbial growth in keropok lekor has significantly increased, showing that cross-contamination has occurred during keropok lekor processing. Besides, during food handling, the microorganism has access to the raw materials in many ways, such as environment, equipment used, food handlers during processing. Nor-Khaizura, Hassan, Bakar, and Rusul (2009) reported that the food handling process will change the product's microbiological status. Even though boiling in the processing method can decrease microbial levels, recontamination can still occur during post-processing (cooling) and food handling.

### 2.4.2 Street food Satar

In Malaysia, the dish known as "satar" is considered a heritage dish, and it is typically served as an appetizer at any celebration on the East Coast region, particularly in Kelantan and Terengganu. It is regarded as an important section of the tourism business because it helps attract visitors to a place. Heritage food is an element of heritage tourism, which is considered an essential area of the tourism industry. Satar, which is cooked over a charcoal fire, is a mixture of spices and minced fish wrapped in banana leaf and grilled until cooked and ready to eat. Local people often used red jobfish (Pentapodus, Nemipterus, and Scolopsis), yellowstripe (Selaroides leptolepis) scad, and Spanish mackerel (Scomberomorus spp.) to make delicious satar (Ramli, Lani, Ibrahim, Alias, & Hassan, 2014).

Satar is also referred to as street food since they are prepared, cooked, and sold by the roadside, for instance, consumption or consumption later without the need to make any additional (Lani, et al., 2015). Satar is a fish-based street food famous in Terengganu but can also be found in Kelantan night market. In Terengganu, satar is a cheap, tasty, and handy food containing essential nutrients. It is also affordable and delicious. Satar is a snack that is typically provided at teatime on street food. The originality of this cuisine and the scent of the banana leaf and other ingredients after they have been grilled have attracted street food consumers who have tried and enjoyed it. Satar is a local delicacy that can be found practically anywhere in Terengganu.

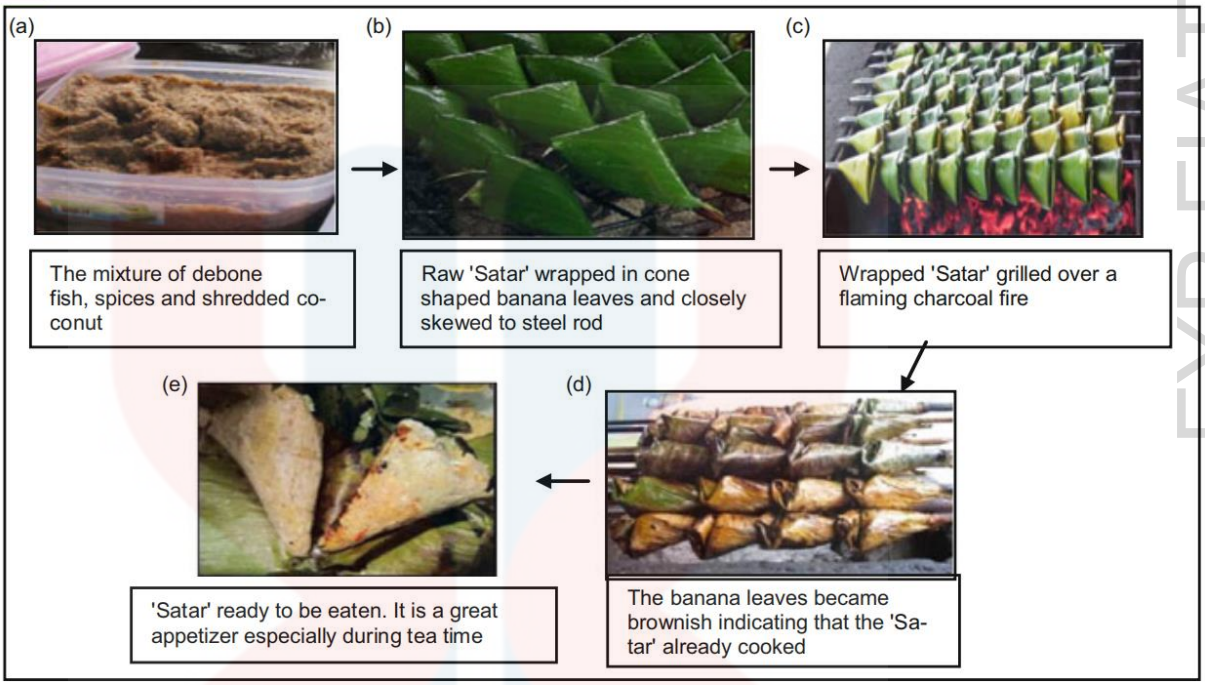



Diagram 2.2 shows the process of making satar in Terengganu, Malaysia (Lani, et al., 2015)

# CHAPTER 3

# MATERIAL AND METHODS

## 3.1 Chemical and media

HiMedia Nutrient Agar (NA) (Mumbai, India), HiMedia Salmonella-Shigella (SS) Agar (Mumbai, India), HiMedia Bacillus cereus (BC) Agar (Mumbai, India), OXOID Eosin Methylene Blue (EMB) Agar (England), Polymyxin B Selective Supplement (FD003) and Egg Yolk Emulsion (FD045), 70% ethanol, and 100% ethanol.

### 3.2 Apparatus and equipment

Dilution bottle (1000ml, 500ml, and 250ml), petri dish, stomacher bag, bag mixer (Interscience:P021230511094), pipette (Transfarpette:18F52060), pipette (Thermoscientific:QZ74845) , hockey stick, bunsen burner, colony counter, digital balance, incubator (Jeiotech: IB-21F SO79161), beaker (1000ml, 500ml, and 250ml), wire loop, autoclave (Hirayama hve-50: 30614046657), measuring cylinder (1000ml, 500ml, 250ml, 50ml, and 10ml), blue tip, yellow tip, blue tip box, yellow tip box, conical flask (250ml), test-tube, test-tube rack, laminar airflow (Azteclab scv-4A1: 2007-27410) and light microscope (Rex vision Y100: 000056).

### 3.3 Preparation of media agar

The media is prepared according to the manufacturing protocol. 28g of Nutrient agar was suspended in 1L of distilled water. Then, thoroughly mix and dissolve the solution on the hot plate in a beaker. The dissolve solution is sterilized in an autoclave for 15 minutes at 121$^{o}$C. The media solution is cooled down until the temperature reaches 50$^{o}$C. After that, 20ml of the media solution is poured into the sterile petri dish and waited until the solution solidifies at room temperature (HiMedia Lab, 2021). The dry medium must be stored at 10-30$^{o}$C if needed and used before the expiry date mentioned on the

packaging. The nutrient agar must be stacked upside down, screened around the petri dish with parafilm, and stored in a refrigerator at 4°C.

The step above is repeat using different type of selective media followed the manufacturing protocol. SSA (HiMedia Lab, 2018) was prepared using 63.02g of SSA powder into 1L of distilled water. However, the SSA solution was not required for autoclave sterilization instead of using Bunsen burner to dilute the agar. Next, EMBA (OXOID, 2010) was prepared using 37.5g of EMBA powder into 1L of distilled water. Finally, BCA (HiMedia Lab, 2019) was prepared using 41g of BCA powder into 1L of distilled water. After cooling, BCA solution was added with rehydrated Polymyxin B Selective Supplement (FD003) and 25 ml of sterile Egg Yolk Emulsion (FD045) aseptically.

### 3.4 Sample collection

Samples of fish satay were obtained directly from three different street vendors in Jeli, Kelantan. The serving utensils of each food vendor were used to collect approximately 50g of each sample. The samples were collected aseptically and labeled before being placed in a pre-sterile zip lock bag. After that, the samples were transported to the laboratory, where they were analysed.

### 3.5 Microbiological analysis method

Standard Plate Count (SPC) method is used to estimate the population of bacteria in a food sample. Tenfold replicate serial dilution is prepared from $10^1$ to $10^6$. The stomacher machine was used to properly mix the food sample with peptone water. The conical flask was filled with the sample solution, and the remaining six test tubes were filled with sterile peptone water. Drawn 1ml of sample into the sterile pipette and transferring to a first test tube for a total volume of 10 ml was completed. An initial dilution of $10^1$ is presented. The test tube was shaken to ensure that the sample solution and diluent were thoroughly incorporated. The old pipette tip was removed and replaced with a new one. A new pipette tip was used to transfer 1 ml of sample solution from the $10^{-1}$ dilution into the second tube of $10^2$. 1 ml from the previous tube to the following 9 ml diluents was repeated for the remaining tubes. The final test tube will have a dilution of $10^6$ for the bacteria/cells (1 in 1,000,000).

A 0.1ml sample solution from a test tube was pipetted onto the center of an agar plate's surface. After that, the hockey stick is dipped in alcohol and flamed over a bunsen burner to sterilize it. The sample was evenly spread onto the surface of the agar using a sterilized hockey stick. While the sample is being spread, the petri dish underneath is rotated. The petri dish was labeled and incubated all the plates at 37°C for 24 hours. This process was repeated with different selective media agar using SS Agar, BC Agar and EMB Agar. For the result, monitor the total growth of bacteria and identify the type of bacteria present in the food sample.

The result obtained was counted using a plate counter on each petri dish. The colony-forming unit (CFU) is an indicator of viable cells of the bacteria or the fungus.

Dead and live cells are counted in direct microscopic counts, but only viable cells are measured. The findings are indicated for the convenience of liquids as CFU/mL (colony units per milliliter) and solids as CFU/g (colony-forming units per gram.). It can be calculated using the below formula:

$$CFU/mL = \frac{number\ of\ colonies\ \times dilution\ factor}{\text{the volume of a culture plate}}$$

### 3.6 Gram staining method

Gram staining is a method for identifying the distinct cell wall components of two big groups of bacteria between gram-positive and gram-negative, for gram staining method, using a clean and sterilized slide. Smear a loopful of bacterial samples on a clean slide. Then, heat fixation of the slide using a Bunsen burner allows air drying. Poured 3 to 4 drops of crystal violet reagent onto the slide and let it sit for 1 minute. Rinse the crystal violet with tap water. Poured the gram iodine about 3 to 4 drop cover on the slide and let it sit for 1 minute. Rinse the gram iodine with tap water. Then, drop 3 to 4 alcohols on the slide for decolorization. After 30 seconds, rinse with water. Add a few drops of safranin, let it sit for 1 minute, and rinse with water. Then, use a paper towel to dry up the slide. Use a light microscope and oil immersion to examine the staining results. For negative gram staining, the result shows pink/red color meanwhile, for positive gram staining, the result appeared purple/blue color (Strasbourg, 2019).

# CHAPTER 4

## RESULT AND DISCUSSION

The microbiological analysis of selected street food (fish satay) samples in Jeli Kelantan was performed in this study, and the results show in Table 3 below. The food sample is taken directly from the street vendor at three different places, and the result shows that all the food samples are contaminated with various types of bacteria, such as *Salmonella spp., Shigella., E. coli, and Bacillus cereus.*

The Total plate Count (TPC) method is used in this study to estimate the total growth of microorganisms using the pour plate method on nutrient agar. Kokkinakis et al. (2020) stated that the TPC method is a general test that counts numerous organisms that grow under aerobic circumstances at mesophilic temperatures on a specified growth medium. This test gives significant information for assessing food safety and quality. After 24 hours of incubation in a 30˚C incubator, the plate is observed for bacteria growth. The result is tabulated in Table 3 using mean colony-forming unit (CFU/ml) and standard deviation (SD).

Table 4.1 Total bacterial count of fish satay in Jeli, Kelantan

| **Food sample** | **Nutrient agar ($10^7$ CFU/ml)** | **Salmonella shigella agar ($10^4$ CFU/ml)** | **Eosin methylene blue agar ($10^5$ CFU/ml)** | **Bacillus cereus agar ($10^5$ CFU/ml)** |
|---|---|---|---|---|
| FS1 | 2.5 ± 3.5 | n.d. | n.d. | 3.50 ±1.13 |
| FS2 | 13.0 ± 2.83 | 6.0 ± 2.83 | 1.62 ± 1.96 | tntc |
| FS3 | tntc | tntc | tntc | tntc |

*n.d. refer to not detected

*Each data point represents mean ± SD (standard deviation) of replicates sample

## 4.1 Total bacterial count of Fish satay



The total bacterial contamination found in the food samples (fish satay) tested was high as shown in Table 3. The total colony growth in nutrient agar in sample FS1 is $2.5\times10^7$ CFU/ml. Next, no single colony is detected in Salmonella Shigella agar and eosin methylene blue sample FS1. There is colony growth detected in *Bacillus cereus* in sample FS1 with a total $3.50\times10^5$ CFU/ml colony. The total colony growth in nutrient agar in sample FS2 is $13.0\times10^7$ CFU/ml. Then, there is colony growth in Salmonella Shigella agar in sample FS2, which is $6.0\times10^4$ CFU/ml. The total colony growth in eosin methylene blue agar is $1.62\times10^5$ CFU/ml, and colony growth in Bacillus Cereus agar

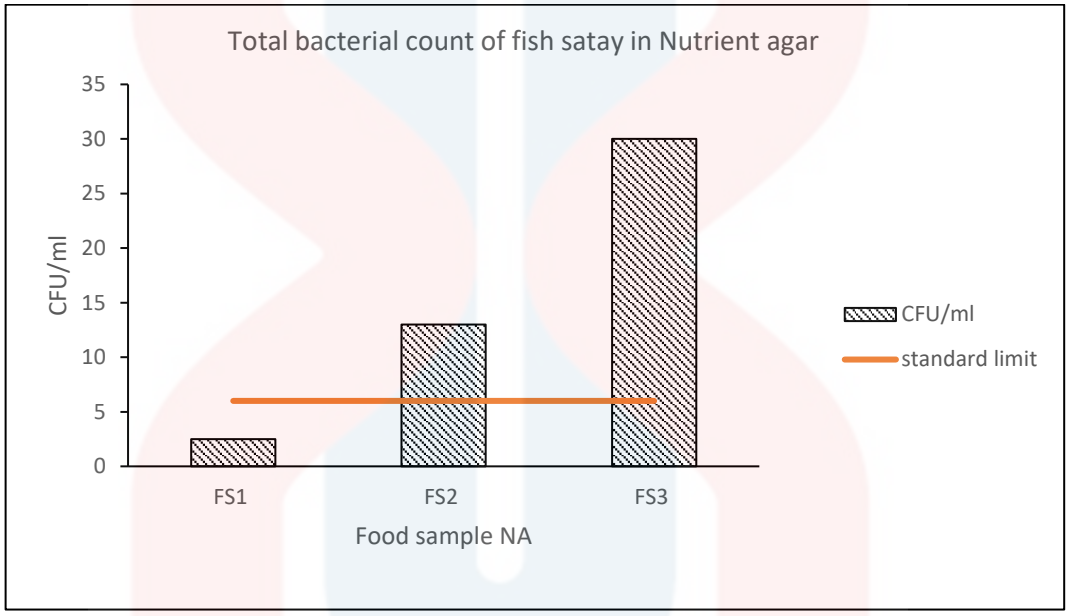
contains more than three hundred (>300) labeled as tntc (too numerous to count). However, in FS3, the total colony growth in nutrient agar, Salmonella Shigella agar, eosin methylene blue agar, and Bacillus Cereus agar had exceeded 300 and labeled as tntc.

### 4.1.1 Total Plate Count (Nutrient Agar)

The total bacteria of all food samples examined in this study was contaminated with various bacteria, which means there is a risk of getting a foodborne illness. The nutrient agar is used to grow bacteria that can be used to analyze food samples. The total bacteria for FS1 are $2.5 \times 10^7$ CFU/ml lower than FS2 is $13.0 \times 10^7$ CFU/ml. However, the total bacteria for FS3 are tntc because the number of colonies grew more than three hundred (> 300), making it difficult to count. Diagram 1 below shows the colony of bacteria growth on nutrient agar. The colony growth in the nutrient agar has different colors, which means there is various bacteria growth. Most bacteria colonies that form in the nutrient agar are white, pale yellow, and slightly brown. The form of bacteria growth is circular with flat elevation.

A previous study reported that total bacteria ($\geq 10^5$ CFU/g) were considered unsatisfactory based on Food Act 1983 and Regulation 1985 (See, 2016). Moreover, based on Microbiology Guideline for Food (2014), the total bacteria ($\geq 10^7$ CFU/g) are considered unsatisfactory. Thus, the total bacteria for all food samples fish satay (FS1,

FS2, and FS3) exceeded the limitation of $\geq 10^7$ CFU/g did not follow the food safety standard.




*The 30 CFU/ml indicates tntc (>300) of bacterial count

Figure 4.1 shows total bacteria count of fish satay in Nutrient agar ($10^7$ CFU/ml)

Sources: Microbiology Guideline for Food (2014)

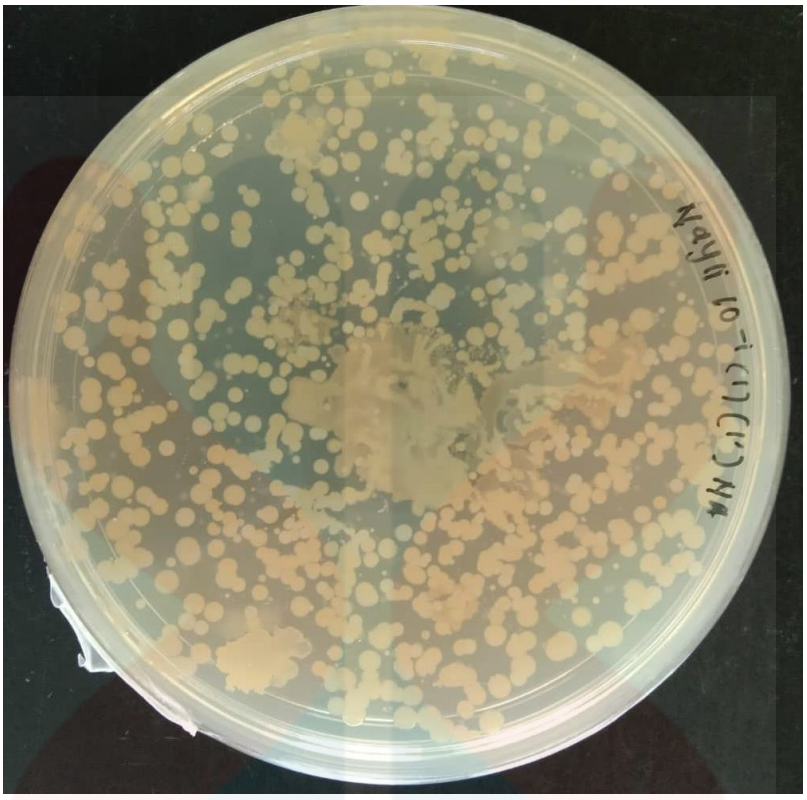


Figure 4.2 shows bacteria growth on nutrient agar

The Gram staining method is used in this study to determine the microorganism growth on an agar plate. The Gram stain is essential for determining the bacterial phenotype. The staining process distinguishes bacteria based on the structure of their cell walls. Gram-positive cells contain an abundant coating of peptidoglycan on their surface, which gives them a blue to purple coloration. The peptidoglycan coating on Gram-negative cells is quite thin, and they appear reddish-pink (Smith & Hussey, 2018). Then light microscopic is used to examine the gram stain results. This method can identify the bacteria's shape and size and their clumping patterns. Diagram 4.2 shows the gram-positive streptococcus bacteria. The bacteria color shown is purple under the microscope because of their thick peptidoglycan membrane, keeping the crystal violet dye pigment.

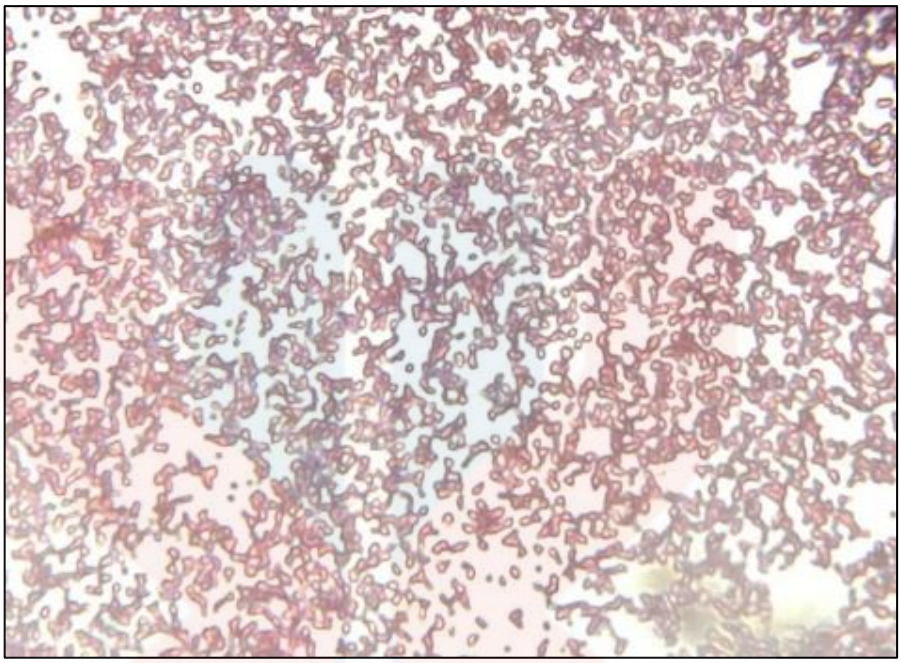
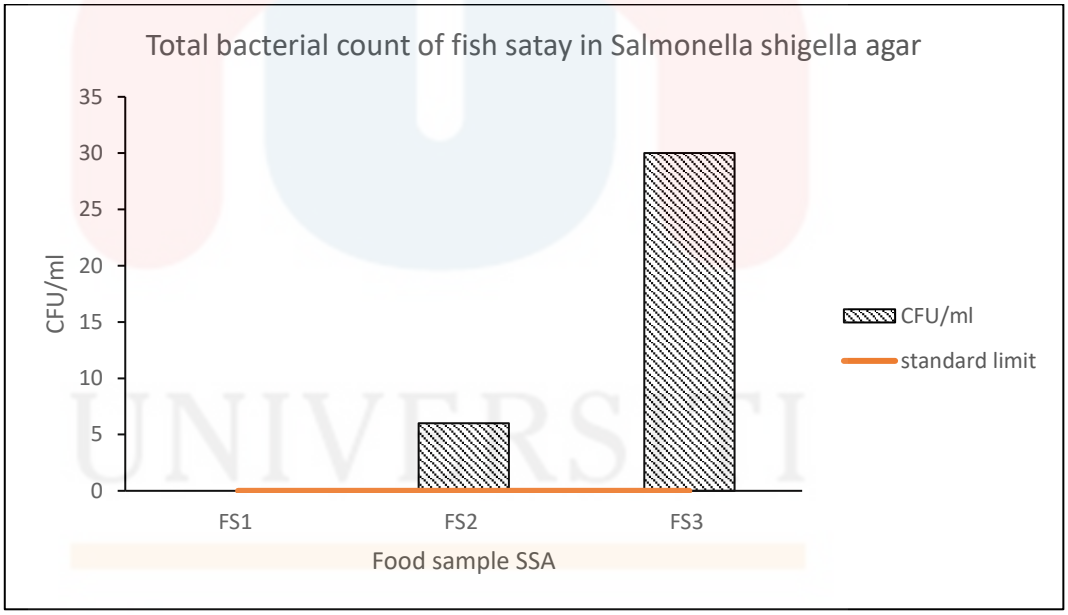


Figure 4.3 show *Streptococcus* bacteria growth on Nutrient agar

### 4.1.2 Total bacterial count in Salmonella Shigella agar

Based on Table 3, the total bacteria count in Salmonella Shigella agar is detected in samples FS2 and FS3 but not in sample FS1. Salmonella Shigella agar is a selective media that can detect *Salmonella spp.* and *Shigella spp.* or other bacteria from a food product, pathological specimen etc. Mesophilic and facultatively anaerobic Gram-negative *Salmonella spp.* are rod-shaped bacteria that do not produce spores and move about freely. Environmental *Salmonella spp.* may persist over lengthy periods (Odo, Uchechukwu, & Ezemadu, 2021). According to technical data by Himedia Laboratories, there are more than one species of bacteria that can grow on Salmonella Shigella agar.

Based on Diagram 2 below, the black colonies indicate *Salmonella spp.* bacteria such as *Salmonella choleraesuis*, *Salmonella Typhi*, *Salmonella Typhimurium*, or *Salmonella Enteritidis* bacteria species. Meanwhile, the pink colonies show *Escherichia coli* and *Enterobacter aerogenes* bacteria species. There are also colorless bacteria growth on Salmonella Shigella agar, *Shigella flexneri* species. According to the Compendium of Microbiological Criteria for Food (2018) and Food Safety and Authority of Ireland (2020), if *Salmonella spp.* is detected in 25g of food sample, it is categorized as potentially hazardous.




*The 30 CFU/ml indicates tntc (>300) of bacterial count

Figure 4.4 shows total bacteria count of fish satay in Salmonella shigella agar ($10^4$ CFU/ml)

Source: Compendium of Microbiological Criteria for Food (2018) and Authority of Ireland (2020)

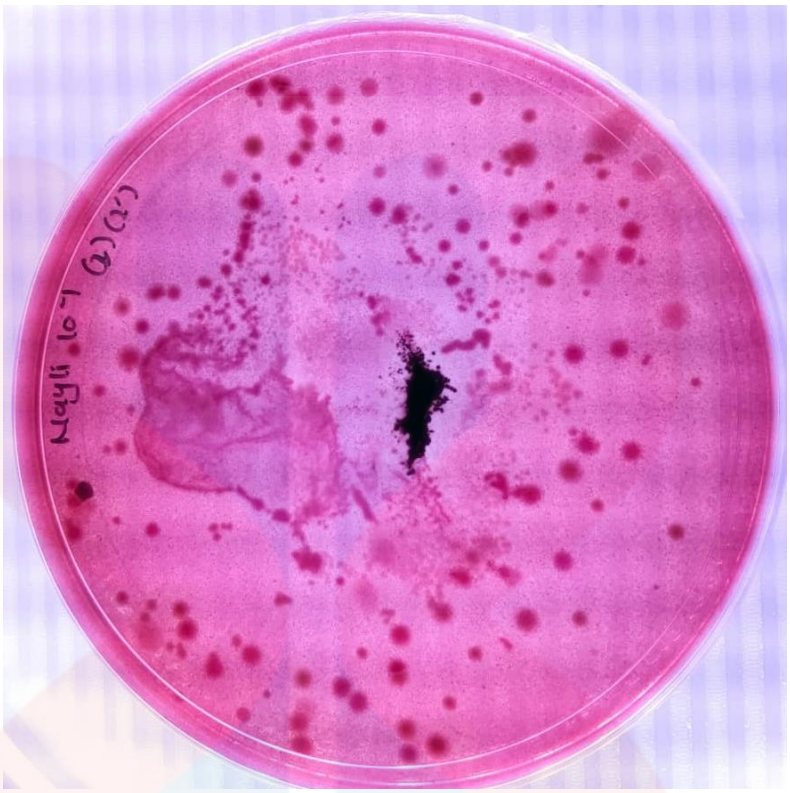


Figure 4.5 shows bacteria growth on Salmonella Shigella Agar

*Salmonella spp.* bacteria are a bacterium that may cause a wide range of illnesses in humans. According to Kumar, Datta, and Lalitha (2015), the natural hosts for *Salmonella spp.* are generally animals, birds, and humans. Fish may not be a natural host for *Salmonella spp*. However, it is well established that the growth and multiplication of *Salmonella spp.* in food environments primarily depend on parameters including temperature, pH, availability of vital nutrients, contact surface, food matrix, and water activity.

*Salmonella spp.* can live and be found in seafood even after it has been frozen for an extended period. According to the stall's owner, fish satay is stored at the freezing temperature but not at the stall. When the food sample (fish satay) was bought directly from the stall, it was already at room temperature, meaning the sample was not kept at freezing temperature, leading to *Salmonella spp.* growth. *Salmonella spp.* is most found

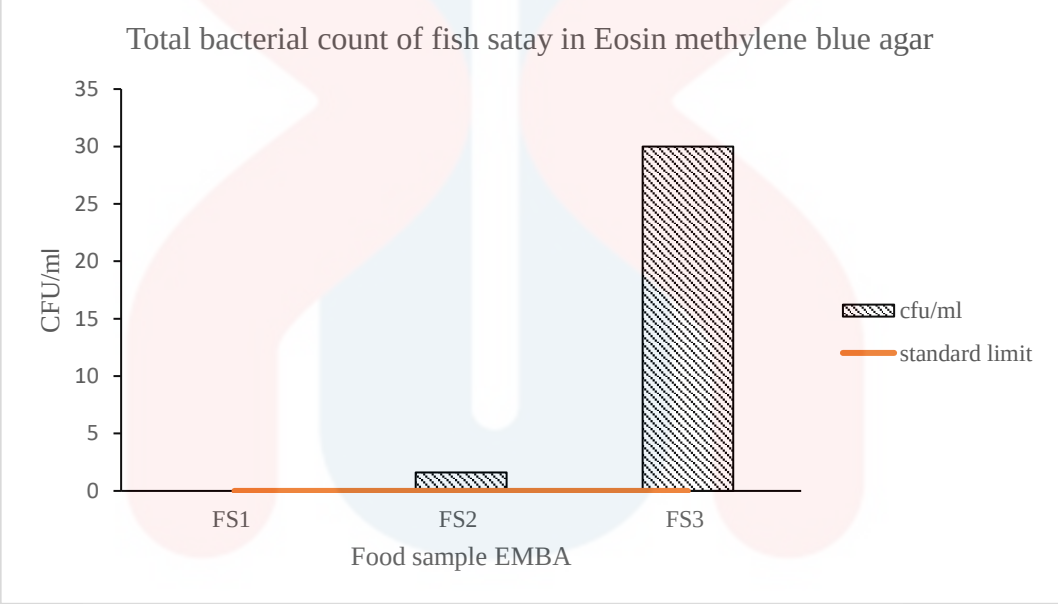
in raw or undercooked food, but it is easily controlled and can be killed by cooking the foods at high temperatures. Cleanliness and sanitation are key in keeping the food free of *Salmonella spp.* contamination.

### 4.1.3 Total bacterial count in Eosin methylene blue agar

Eosin methylene blue agar is used to isolate *Enterobacteria*. The agar used to grow enterococci, staphylococci, and yeast, all gram-positive bacteria and non-pathogenic, non-lactose-fermenting microbes, can also grow on that EMB agar. Diagram 3 below shows black color bacteria growth on EMB agar appear to be *Escherichia coli* bacteria. The characteristic of *E. coli* bacteria that growth on EMB agar is a black colony with a green metallic sheen on the backside. Based on Table 3 above, *E. coli* is not detected in sample FS1, where the agar plate is clean and didn't grow any microorganism. However, *E. coli* present in sample FS3 is higher than sample FS2, where the bacteria grew more than 300 colonies (tntc).

The *E. coli* bacteria growth on sample FS2 is $1.62\times10^5$ CFU/ml. It shows that food sample FS3 is more contaminated than food sample FS1 and FS2. According to the Compendium of Microbiological Criteria for Food (2018), if *E. coli* is detected more than ($> 10^2$), categorized as unsatisfactory. When the total of *E. coli* colonies grows in food sample less than 3 (< 3), it will be classified as satisfactory. However, according to Food Act 1983 and Food Regulation 1985, *E. coli* that are detected in 25g of food sample are

not accepted. A bacteria *Escherichia coli* (*E. coli*) can be found in various places, including the environment, food, and the intestines of humans and animals. *E. coli* bacteria may cause diarrheal, urinary tract infections, lung diseases, pneumonia, and other ailments ( Mueller & Tainter, 2021).




*The 30 CFU/ml indicates tntc (>300) of bacterial count

Figure 4.6 shows total bacteria count of fish satay in Eosin methylene blue agar ($10^5$ CFU/ml)

Source: Compendium of Microbiological Criteria for Food (2018) and Authority of Ireland (2020)

When it comes to foodborne pathogens, *E. coli* is the most well-known. This bacterium has been identified as the source of a significant health hazard associated with eating contaminated food (Thi et al., 2021). The presence of these bacteria, which are considered hygienic indicators, indicates that hygiene standards have been violated

during or after food production. Previous studies reported that *E. coli* foodborne are common in Malaysia and cause diarrheal diseases. They also found the cause of infection of *E. coli* on street food is the utensils were in poor sanitary condition, raw materials were improperly stored, storage temperatures were abused, and meals were exposed at temperatures and times that were not appropriate.

According to World Health Organization (WHO) (2018), *E. coli* is a bacterium that may cause food poisoning among consumers. It primarily spreads to humans when they eat food that has been contaminated, like raw or undercooked food. The symptoms of food poisoning, such as abdominal pain, fever, vomiting, and diarrheal, sometimes lead to bloody diarrheal. *Escherichia coli* belongs to the Enterobacteriaceae family, is used as a hygiene indicator to test the quality of a food product and requires a high temperature to be destroyed (Health Protection Agency, 2009). Thus, it suggested the fish satay must be appropriately cooked at a high temperature to ensure the bacteria are killed and prevent food poisoning.

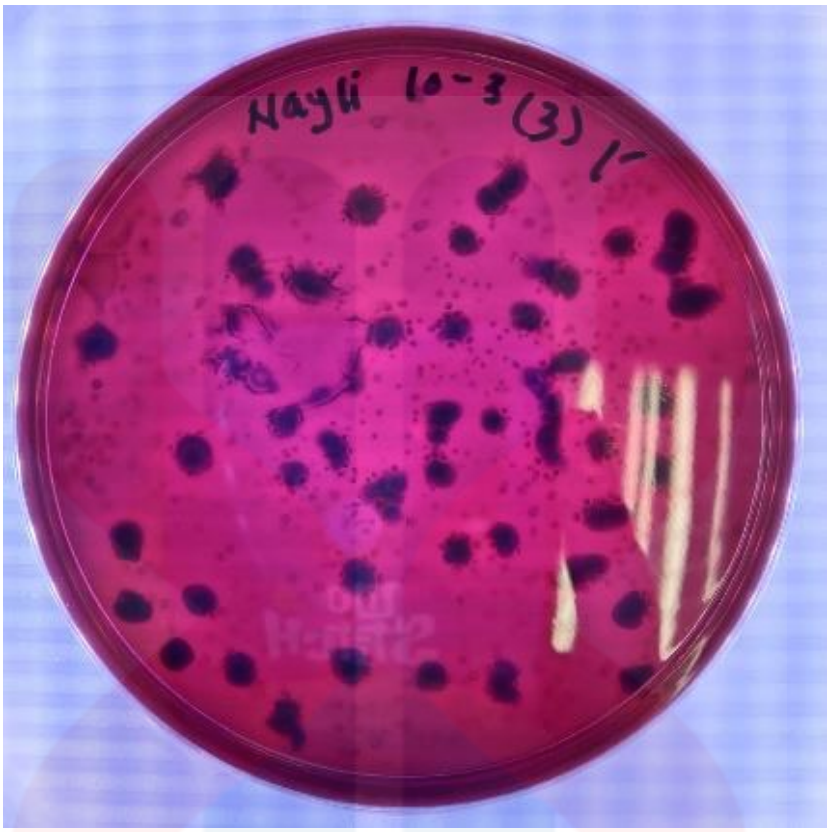
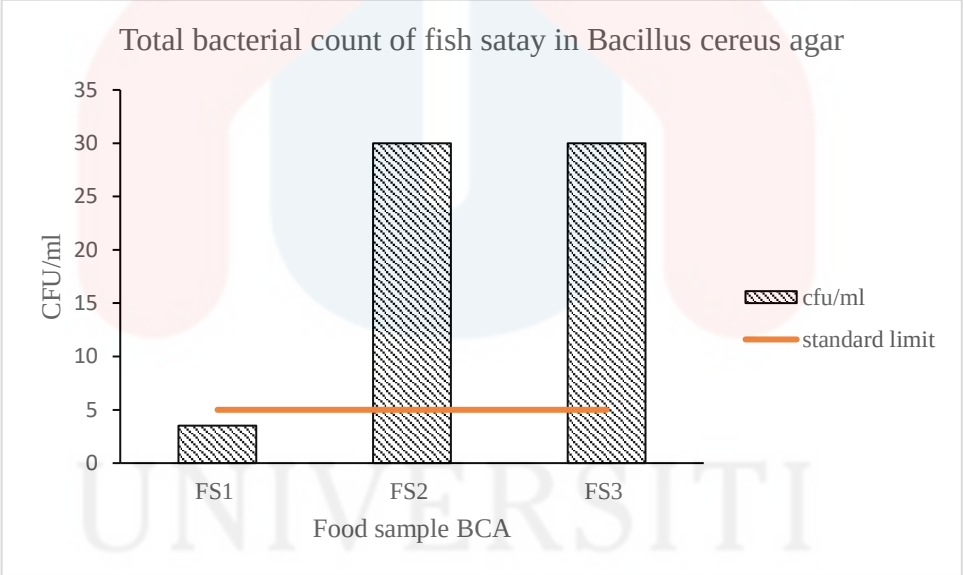



Figure 4.7 shows bacteria growth on Eosin Methylene Blue agar

### 4.1.4 Total bacterial count in Bacillus cereus agar

*Bacillus cereus* is a gram-positive and aerobic spore-forming bacteria found in soil, on vegetables, and in many raw and processed foods. *Bacillus cereus* is a bacterium found in soil, on vegetables, and in many raw and processed foods (Bottone, 2010). Food poisoning caused by *Bacillus cereus* can develop when meals are prepared and then left out without appropriate refrigeration for several hours before being served for consumption. Based on the table result, *Bacillus cereus* bacteria are present in all three samples. The total number of colonies of *Bacillus cereus* in FS1 is $3.50\times10^5$ CFU/ml.

Meanwhile, FS2 and FS3 total colony exceed 300 considered as tntc (too numerous to count). The result shows that sample FS2 and FS3 is more contaminated than FS1. According to the Compendium of Microbiological Criteria for Food (2018), the accepted level of *Bacillus cereus* in food samples is between $10^3 – 10^5$ CFU/ml, and the level of *Bacillus cereus* is more than greater $10^5$ is considered unsatisfactory, which is not accepted. Thus, all the food sample FS1, FS2 and FS3 is not accepted because the number of CFU/ml is higher than the accepted level.




*The 30 CFU/ml indicates tntc (>300) of bacterial count

Figure 4.8 shows total bacteria count of fish satay in Bacillus cereus agar ($10^5$ CFU/ml)

Source: Compendium of Microbiological Criteria for Food (2018)

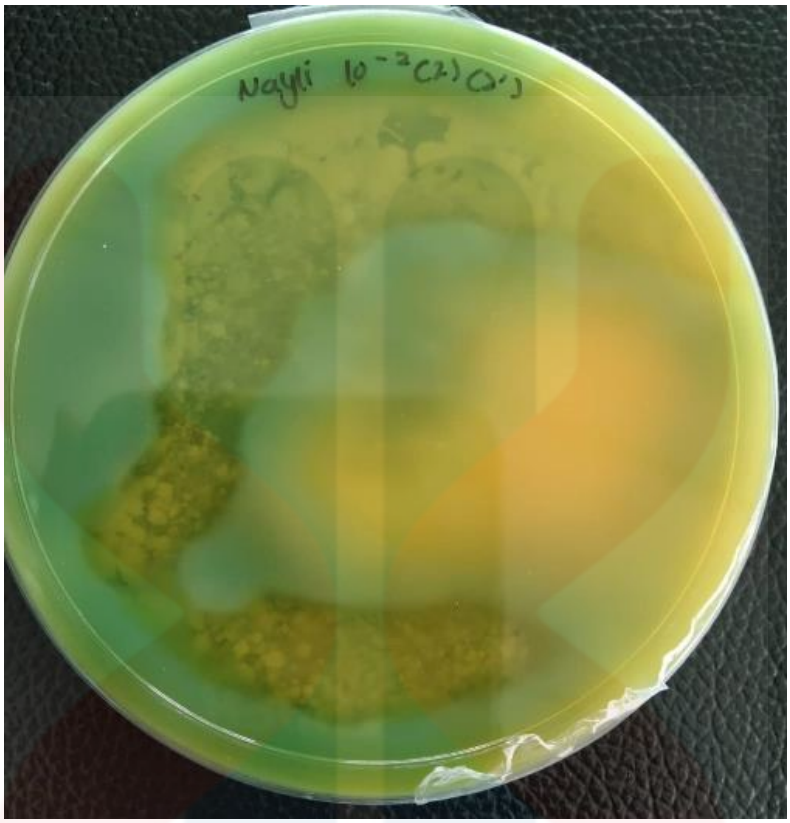


Figure 4.9 shows bacteria growth on Bacillus Cereus agar

Diagram 4 above shows *Bacillus cereus* bacteria growth on Bacillus Cereus agar. However, there are different colors of bacteria growth: yellowish and bluish colors that represent different types of bacteria growth. The bacteria's bluish represent Bacillus cereus and E. coli; meanwhile, the yellowish color represents *Proteus vulgaris* bacteria. According to Rana, Panda, Pathak, Gupta , & Thakur (2020), diarrheal and emetic symptoms are caused by *Bacillus cereus*, the bacteria that causes food poisoning. Diarrheal enterotoxin is produced in the host's digestive tract by bacteria consumed with contaminated food. Emetic syndrome is a condition that causes people to vomit because they eat food that already has an emetic toxin in it.

## 4.2 General discussion

The result shows that the street food in Jeli, Kelantan are contaminated by different types of bacteria. There are issues and concerns related to street food that can cause foodborne diseases to the consumer. A previous study reported that high levels of bacteria do not necessarily suggest that a food is dangerous on its own; instead, they reflect improper handling of food, improper storage, poor personal hygiene, or a lack of general cleanliness (Shamimuzzaman, et al., 2022) and (Bi, et al., 2018). Table 3 shows that food sample FS3 is more contaminated than FS1 and FS2. I have noticed that the texture for food sample FS3 is slimmer and stickier than sample FS2, but for sample FS1, the food is still cold, and a bit dried. There is a chance that samples FS2 and FS3 are contaminated at the stall during food handling.

The storage condition is essential to ensure the safety of street food. The food sample (fish satay) were placed in an open box on the table with other frozen food. The open box could attract flies and insects because of the fishy odor from fish satay. According to Latchumaya *et al*. (2021), when foods are displayed in an open setting without being properly incubated, there is a great likelihood that bacterial contamination may develop. Rather than having a permanent location to store their raw ingredients, many street food sellers stored them at home and brought the next day, without proper coverage, to their stall locations (Proietti, Frazzoli, & Mantovani, 2014). As a result, food in transit is more likely to get contaminated. Someplace left the food in plastic and hanging onto the stall can cause the temperature of the food to rise and allow the bacteria to grow.

Fish satay is a frozen food that needs to store at a temperature of -18˚C or below to inhibit the growth of microorganisms. Besides, stored at freezing temperatures can preserve frozen foods' nutritional content and freshness where spoilage bacteria cannot carry out their activity (Shamimuzzaman, et al., 2022). Thus, if the foods were kept at ambient temperature for more than 4 hours, it could develop high bacteria counts, putting public health at risk. This could be the reason for microbial growth in food sample fish satay as the operation time of the street food stall start at 9.00 am until 10.00 pm. Therefore, the street food fish satay must be kept at a low temperature in a closed container to avoid cross-contamination and inhibit microbial growth.

Contamination can occur directly from food handlers that are not aware of food and personal hygiene. The food handlers at the street food sample are not wearing suitable clothe and attire while handling the food products. They are not wearing an apron, gloves, hair cover, and shoes essential for food handlers. They also use their bare hand when handling unhygienic food products. Food handlers do not always use correct food processing and handling procedures is a cause for worry since the high microbiological counts show that these practices are not always followed (Moloi, Lenetha, & Malebo, 2021). A previous study from Ariffin et al., (2019) reported that the street food at Chow Kit, Kuala Lumpur, can cause foodborne diseases to the consumer as the food handlers did not follow food hygiene practices. Another possible cause of contamination is poor hand hygiene among vendors, many of whom handle raw food, collect money, and package cooked food without washing their hands. Fd handlers' attitude is the most important factor (New, et al., 2017).

It is necessary to coordinate the attitudes of food handlers to guarantee that food safety is practiced. Food handlers must be aware of having food hazards in their daily meal and why it is crucial to maintain food security. Rane (2011) reported that food

infections, including Escherichia coli and Salmonella spp. are spread via unsanitary street food handling by certain vendors to customers, and those bacteria can survive on fingertips and other surfaces for long periods, even after cleaning. Thus, it is important for food handlers always to wash their hands to prevent contamination of the food sold to customers. Nikiema et al. (2021) stated that it is important to follow food hygiene practices on street food as it generated vital concern to prevent food poisoning outbreaks among a large group of people.

Bacteria substances can be transferred from one food to another via contaminated surfaces or utensils if proper washing and disinfection are not performed. Cross-contamination occurs most frequently when a handler uses the same cutting boards or kitchen tools to process raw and cooked foods. When samples were bought from the street vendor, the food handlers used the same plate to place raw and cooked food without using proper tools and equipment. There is also no clean water supply that can be used for cleaning. The running water is rarely accessible at stall locations. Thus, utensils and dish cleaning are frequently performed in stagnant waters in buckets and occasionally without soap, resulting in contamination of the utensils. This scene supported by a research study of Mangaung Metropolitan Municipality that most vending outlets did not have running water; instead, people washed their hands and utensils in buckets or bowls. Water is essential for the sanitary condition of street food (Salamandane, Silva, Brito, & Ferreira, 2021). Thus, unhygienic places will attract insects, flies, and pests that can carry infections that are harmful to human health.

Figure 4.10 shows the presence of flies in the storage box of fish satay

The stall location plays an important role in indicating the street food's safety. There are many bacteria growth in food sample fish satay based on the result Table 3 above because of the conditions in which it's made and sold. An increase in foodborne illness could result from food contamination caused by the food vendors' location. Most vendors gather in places where many people could be customers. These conditions facilitate the entry of dust, insects, and exhaust fumes into food, contaminating them and creating a health risk to street food consumers (Were, Were, & Aduol, 2020) and (Birgen, Njue, Kaindi, Ogutu, & Owade, 2020).

Because of their placement on the roadside, the hygiene circumstances in which street vendors prepare and sell their products are frequently unacceptable (Alimi, 2016).

The cleanliness of street food locations is critical to prevent food contamination and customer acceptance. A previous study reported that the cleanliness of street food is the primary factor influencing the perception of Malaysian street food (Abd Hanan, Sadri, & Yusup, 2021). In addition, the consumer in Malaysia prefers clean street food locations. Previous research from Chang, Chu and Shahril (2020) reported that respondents (consumers) prefer to purchase street food in an area that is clean surrounding the stall and away from garbage, wastewater, bathroom facilities, open drains, and animals among other things.


Figure 4.11 shows the location of fish satay stall by the roadside

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

In conclusion, the findings of this study revealed that the food sold on the street at Jeli, Kelantan were contaminated with bacteria. The tabulated result shows that the fish satay is not safe own its own because the total bacterial count does not meet the general microbiological guideline. The various bacteria present on the food sample fish satay can cause foodborne diseases, and the street foods may constitute a significant threat to public health. The most common source of food contamination in street food is when food handlers don't follow adequate hygiene procedures. Bacterial transmission in food is caused by handlers who mishandle the food, not the item itself. Due to food contamination, there is an increased chance for the consumer to be infected with foodborne diseases. However, the bacteria presence could reduce if the food handler follows appropriate food hygiene practice

## 5.2 Recommendation

It is recommended that food handlers be provided with the necessary knowledge and skills to handle food hygienically. This is because high contamination levels in street food products are caused mainly by inadequate food safety training and a general lack of understanding about necessary hygienic standards ( Asiegbu, Lebelo, & Tabit, 2020). The food handlers are responsible for the most crucial aspect of ensuring that food is always safe to consume. Food handlers training programs organized by the Food Safety and Quality Division of the Ministry of Health in Malaysia should be mandatory for all food handlers (Abdul-Mutalib, Syafinaz, Sakai, & Shirai, 2015).

Raise awareness among street food handlers about the importance of personal hygiene and sanitation in the street food industry. The government should develop the Food safety standards for street foods, and monitoring officers should be recruited to ensure that these standards are implemented and adhered to by food handlers and vendors to ensure the safety of street foods. Food vendors/stalls must display their sanitation licenses and health clearances to reassure the public of their cleanliness. Street food owners can obtain sanitation licenses and health certifications for street food vendors through city health inspectors conducting informational drives (Benitez & Olmogues, 2021).

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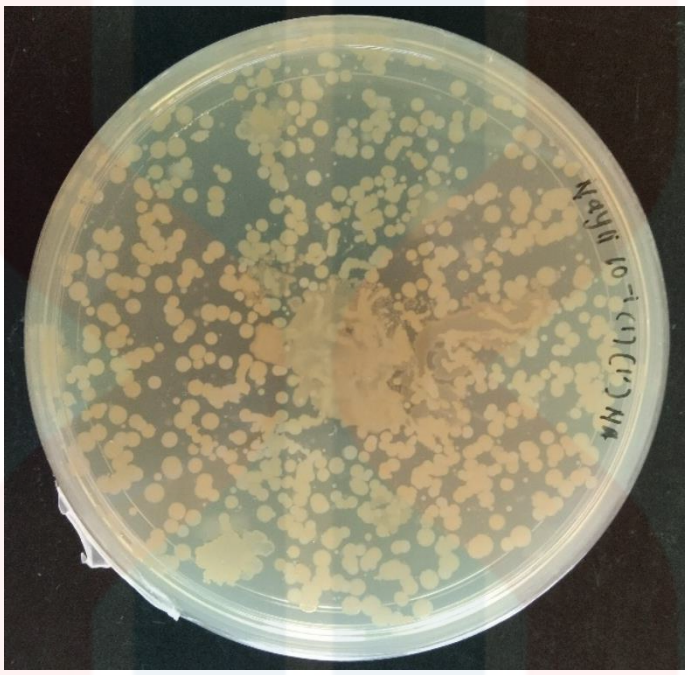
APPENDIX

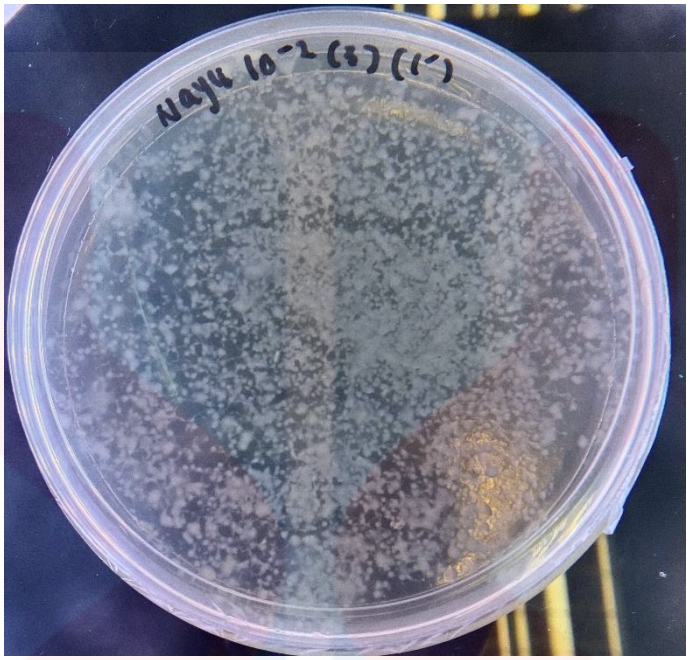
APPENDIX 1

The figure of Nutrient agar and selective agar plate

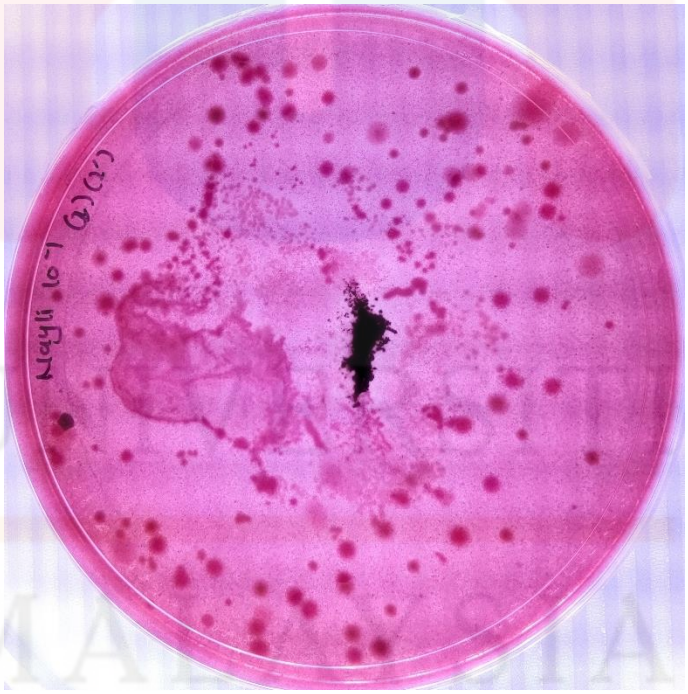


Sample FS1 Nutrient agar

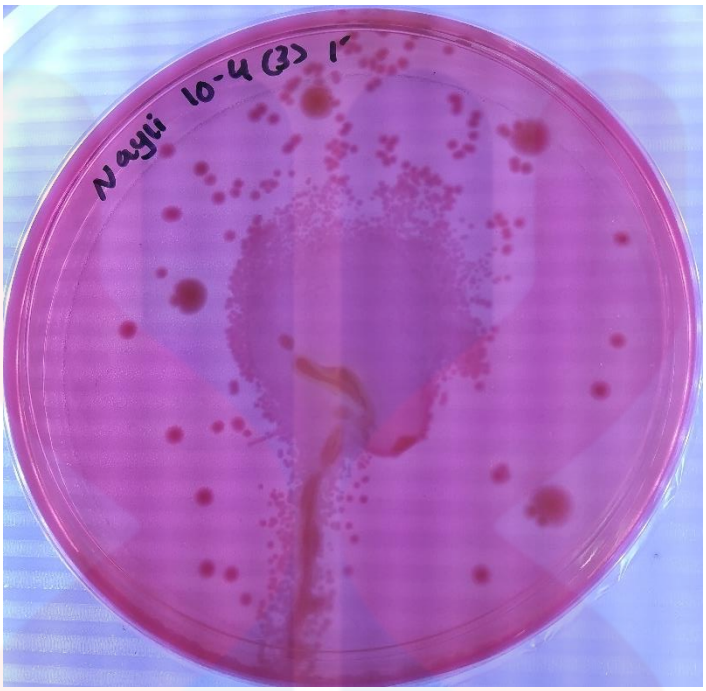
Sample FS2 Nutrient agar



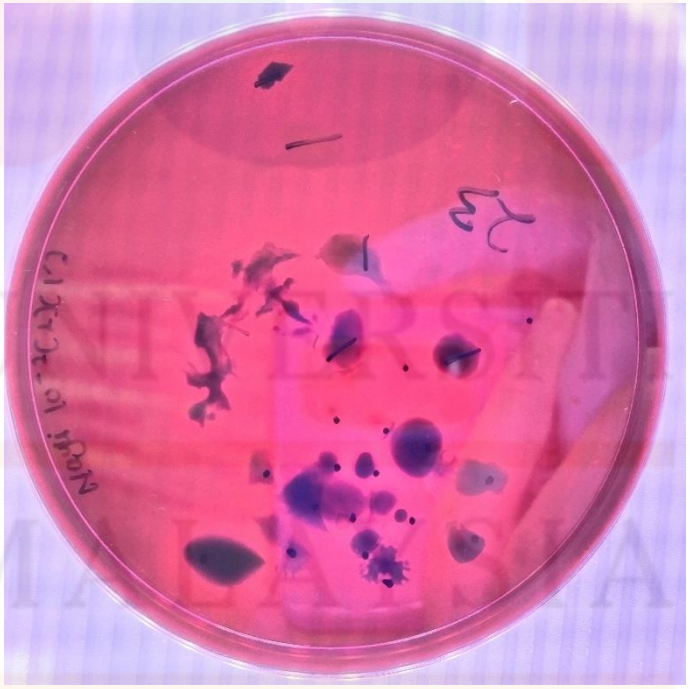
Sample FS3 Nutrient agar



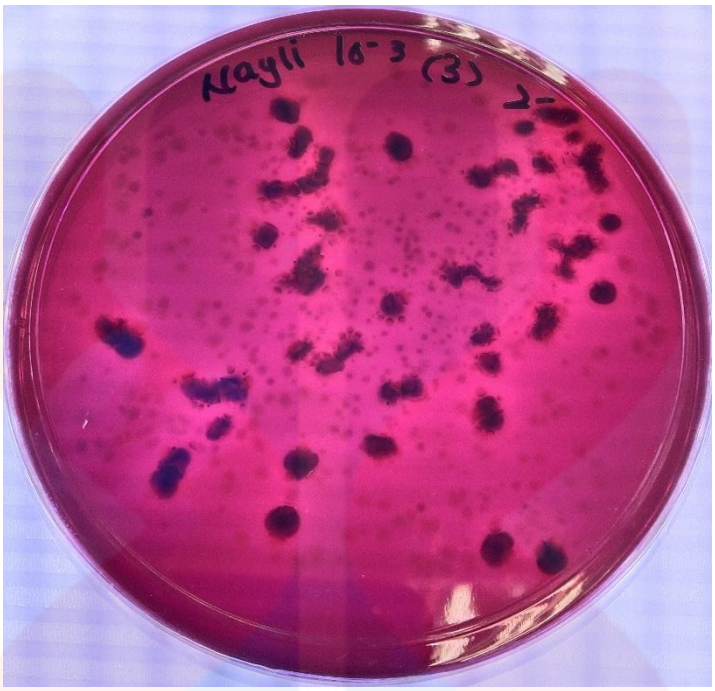
Sample FS2 Salmonella Shigella agar



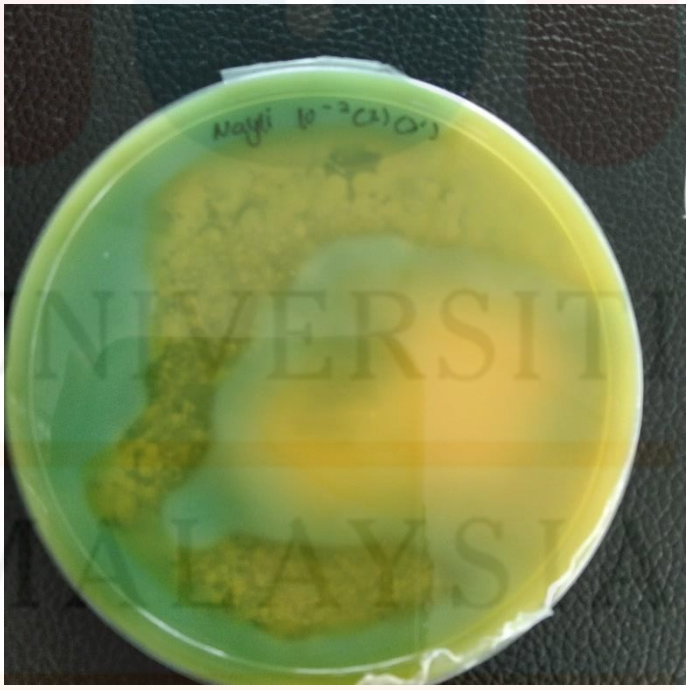
Sample FS3 Salmonella Shigella agar



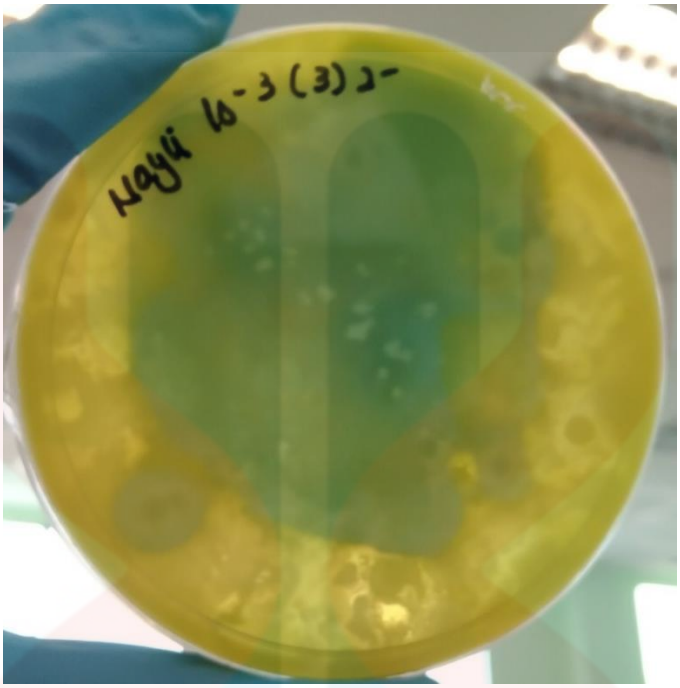
Sample FS2 Eosin Methylene Blue Agar




Sample FS3 Eosin Methylene Blue Agar



Sample FS2 Bacillus Cereus agar



Sample FS3 Bacillus Agar

Figure of fish satay stall in Jeli, Kelantan

Food handlers are not wearing gloves and cooked food placed between raw food

Food handlers not wearing proper attire

Fish satay stall by the roadside near waste area

The table of International Microbial Requirement of Food APPENDIX 3

| | | Satisfactory | Borderline | Unsatisfactory |
|---|---|---|---|---|
| **Malaysia Food Regulation 1985 (Regulation 39) (2014 revision)** | Aerobic colony count | | - | $10^6$ |
| | *E. coli* | | - | n.d. in 1g |
| | *Salmonella* | | - | - |
| | *Bacillus cereus* | | - | - |
| **Food Standards Australia New Zealand 2016 (updated 2018)** | Aerobic colony count | $< 10^4$ | $10^4 < 10^7$ | $\geq 10^7$ |
| | *E. coli* | n.d. in 25g | N/A | Detected in 25g |
| | *Salmonella* | n.d. in 25g | N/A | Detected in 25g |
| | *Bacillus cereus* | $< 10^2$ | $10^2 < 10^3$ | $10^3 \leq 10^5$ |
| **Food Safety and Authority of Ireland (Revision 2020)** | Aerobic colony count | $< 10^6$ | $10^6 < 10^7$ | $> 10^7$ |
| | *E. coli* | n.d. in 25g | N/A | Detected in 25g |
| | *Salmonella* | n.d. in 25g | N/A | Detected in 25g |
| | *Bacillus cereus* | $< 10^3$ | $10^3 \leq 10^5$ | $> 10^5$ |
| **Microbiological Guidelines for Food (Hong Kong 2014)** | Aerobic colony count | $< 10^6$ | $10^6 < 10^7$ | $> 10^7$ |
| | *E. coli* | n.d. in 25g | N/A | Detected in 25g |
| | *Salmonella* | n.d. in 25g | N/A | Detected in 25g |
| | *Bacillus cereus* | $< 10^3$ | $10^3 \leq 10^5$ | $> 10^5$ |