Listeria spp. AND *L. monocytogenes* ISOLATED FROM FOOD PREMISES: PREVALENCE, CHARACTERISTICS, BIOFILM FORMATION AND ANTIBACTERIAL EFFECT OF DETERGENTS

CHEN SOOK NGOH

MASTER OF SCIENCE

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

JANUARY 2024

Listeria spp. AND *L. monocytogenes* ISOLATED FROM FOOD PREMISES: PREVALENCE, CHARACTERISTICS, BIOFILM FORMATION AND ANTIBACTERIAL EFFECT OF DETERGENTS

By

CHEN SOOK NGOH

A dissertation submitted to Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Master of Science

January 2024

ABSTRACT

Listeria spp. AND *L. monocytogenes* ISOLATED FROM FOOD PREMISES: PREVALENCE, CHARACTERISTICS, BIOFILM FORMATION AND ANTIBACTERIAL EFFECT OF DETERGENTS

CHEN SOOK NGOH

Listeria monocytogenes is a ubiquitous foodborne pathogen that can spread through food and food contact surfaces which lead to deadly listeriosis outbreaks, as was recently seen in United States and South Africa. Moreover, *L. monocytogenes* is widely found in food and food products in Malaysia by previous research study. As *L. monocytogenes* adheres to these surfaces and persist in food related environment with development of biofilm before reaching to a host to cause infection. This study aimed to determine the occurrence of *L. monocytogenes*, examine the antibiotic resistance profiles via classification of serotypes and virulent genes and compare *L. monocytogenes* from environment and lab cultured with biofilm study. A total of 322 samples were collected randomly from food industries in Perak from August 2018 to 2019. Prior to classification of serotype, virulence factor and antibiotic resistance research, *L. monocytogenes* was discovered and confirmed using a combination of plating and duplex polymerase chain reaction method. Then, the adherence, biofilm-forming ability, biofilm formation and elimination of environmental isolate were compared to ATCC 19112. Results revealed that 69 samples (21.42%) tested positive for *Listeria* and more than half of these samples (n=41/69, 59.42%)

belonged to serogroup II.2 of *L. monocytogenes* and carried majority of evaluated virulent genes. About 98.48% isolates exhibited resistance against oxacillin followed by penicillin (50%) and clindamycin (45.45%). It was discovered that the type of strain, test surface, temperature and disinfection solutions were variables affecting the development and elimination of biofilm cells. Comparatively, ATCC strain of 19112 developed more biofilm cells than the environmental isolate, but it detached effectively with acid treatment. In conclusion, this study revealed that unhygienic food processing environments promote the growth of biofilm which subsequently contaminate food products and lead to listeriosis arise in Malaysia. Thus, continuous national surveillance programs, monitoring antibiotic resistance in treating *L. monocytogenes* and educating the food handlers with proper cleaning techniques are crucial for preventing the spread of multi-drug resistant *L. monocytogenes*.

ACKNOWLEDGEMENT

Foremost, I would like to express my sincere appreciation and utmost gratitude towards my supervisor, Asst. Prof. Dr. Saw Seow Hoon and my co-supervisor, Asst. Prof. Dr. Yap Moh Lan. Without their patience, motivation and guidance, it would be impossible to complete this project. I truly cherish their unwavering support, priceless counsel and useful recommendations in helping me to overcome obstacles and challenges I faced while conducting the research and when writing the dissertation and journal papers. Besides, I would like to express my deepest gratitude towards UTAR Research Fund of IPSR/RMC/UTARRF/2018-C1/K01 for providing the funding for this research study.

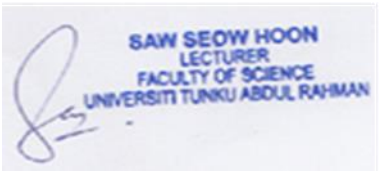
Furthermore, I would like to extend my gratitude and appreciation to my friends, the panel of judges during presentation and laboratory officers for their restless assistance and helpful advice in carrying out this research project. I am particularly appreciative to my lab mates in D213A and D213B, who were always willing to lend a helping hand and offer words of encouragement. Additionally, I would like to convey my sincere gratitude to all food processing industries in Perak who allowed me to enter their food domains for sampling.

Last but not least, I would like to express my sincere appreciation and respect to my family members for their unconditional love, support and understanding throughout this research project. Without their support, I might not have able to get this far.

APPROVAL SHEET

This dissertation entitled "***Listeria* spp. AND *L. monocytogenes* ISOLATED FROM FOOD PREMISES: PREVALENCE, CHARACTERISTICS, BIOFILM FORMATION AND ANTIBACTERIAL EFFECT OF DETERGENTS**" was prepared by CHEN SOOK NGOH and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

Approved by:



_____ Date: 26th January 2024
(Asst. Prof. Dr. Saw Seow Hoon)
Assistant Professor/ Supervisor
Department of Allied Health Sciences
Faculty of Science
Universiti Tunku Abdul Rahman

_____ Date: 26th January 2024
(Asst. Prof. Dr. Yap Moh Lan)
Assistant Professor/ Co-Supervisor
Department of Biological Science
Faculty of Science
Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 26th January 2024

SUBMISSION OF DISSERTATION

It is hereby certified that **CHEN SOOK NGOH** (ID No: **18ADM06536**) has completed this dissertation entitled "*Listeria* spp. AND *L. monocytogenes* ISOLATED FROM FOOD PREMISES: PREVALENCE, CHARACTERISTICS, BIOFILM FORMATION AND ANTIBACTERIAL EFFECT OF DETERGENTS" under the supervision of Asst. Prof. Dr. Saw Seow Hoon (Supervisor) from the Department of Allied Health Sciences, Faculty of Science, Universiti Tunku Abdul Rahman, and Asst. Prof. Dr. Yap Moh Lan (Co-Supervisor) from the Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman.

I understand that University will upload softcopy of my dissertation in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

_____

CHEN SOOK NGOH

DECLARATION

I, **CHEN SOOK NGOH**, hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Name: CHEN SOOK NGOH

Date: 26th January 2024

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
× g	Time gravity
µL	Microliter
µm	Micrometer
β	Beta
bp	Base pair
cfu	Colony-forming units
cfu/ml	Colony-forming units per milliliter
cfu/mm^2	Colony-forming units per millimeter squared
cm	centimeter
g	Gram
h	Hour
hrs	Hours
log_{10} cfu/cm^2	Log_{10} colony-forming units per centimeter squared
log_{10} cfu/ml	Log_{10} colony-forming units per milliliter
mM	Micromolar
mg/L	Milligrams per liter
min	Minute
mL	Milliliter
nm	Nanometer
s	Second
U	Unit
v	Volume
w	Weight

ADI	Arginine deiminases
AgDI	Agmatine deiminases
AMC	Amoxicillin/clavulanic acid
AMP	Ampicillin
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
ATR	Acid tolerance response
BAM	Bacteriological and analytical method
CA	Canada
CAMP	Christie, Atkins, Munch-Petersen test
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
csp	Cold shock protein
d-PCR	Duplex polymerase chain reation
dNTPs	Deoxynucleoside triphosphates
DA	Clindamycin
DNA	Deoxyribonucleic acid
E	Erythromycin
ELISA	Enzyme-linked immunosorbent assay
EPS	Extracellular polymeric substances
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
GDA	Glutamatic acid decarboxylase
HDPE	High-density polyethylene
HI	Hydrophobicity index

HSD	Honestly Significant Difference
IMS	Immunomagnetic separation
ISO	International Organization of Standards
LAB	Lactic acid bacteria
LEB	Listeria Enrichment Broth
LLO	Listeriolysin O
MAR	Multidrug-resistant
MEM	Meropenem
$MgCl_2$	Magnesium chloride
MPN	Most probable number
m-PCR	Multiplex polymerase chain reaction
MWB	Modified Welshimer's broth
NaCl	Sodium chloride
NaOH	Sodium hydroxide
OD	Optical density
OX	Oxacillin
P	Pencillin
PALCAM	Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol
PBS	Phosphate buffer solution
PCR	Polymerase Chain Reaction
PP	Polypropylene
PTFE	Teflon
QACs	Quaternary ammonium compounds
RD	Rifampicin
RNA	Ribonucleic acid
RTE	Ready-to-eat food
S	Streptomycin
SEM	Scanning electron microscopy

SXT	Sulfamethoxazole/trimethoprim
TE	Tetracycline
TMS	Trimethyl silane
TSA	Tryptic soy agar
TSB	Tryptone/Tryptic soy broth
USA	United States of America
USDA	Department of Agriculture in United States
WHO	World Health Organization

E. coli	*Escherichia coli*
hly	Hemolysin
inl	Internalin
L. aquatica	*Listeria aquatica*
L. booriae	*Listeria booriae*
L. cornellensis	*Listeria cornellensis*
L. costaricensis	*Listeria costaricensis*
L. fleischmannii	*Listeria fleischmannii*
L. floridensis	*Listeria floridensis*
L. goaensis	*Listeria goaensis*
L. grandensis	*Listeria grandensis*
L. grayi	*Listeria grayi*
L. innocua	*Listeria innocua*
L. ivanovii	*Listeria ivanovii*
L. marthii	*Listeria marthii*
L. monocytogenes	*Listeria monocytogenes*
L. newyorkensis	*Listeria newyorkensis*
L. riparia	*Listeria riparia*
L. rocourtiae	*Listeria. rocourtiae*

L. seeligeri	*L. seeligeri*
Listeria spp.	*Listeria species*
L. thailandens	*Listeria thailandens*
L. weihenstephanensis	*Listeria weihenstephanensis*
L. welshimeri	*Listeria welshimeri*
R. equi	*Rhodococcus equi*
S. aureus	*Staphylococcus aureus*
Staphylococcus spp.	*Staphylococcus* species
tet	Tetracycline

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, facultative anaerobe, non-spore forming foodborne pathogen that is able to survive under low temperatures of (2-4^{o}C), a wide range of pH conditions (4.5-9.5) and even with high salt content environment up to 40% w/v (Farber and Peterkin, 1991; Liu, 2005, Marian et al., 2012). It is also able to survive under extreme conditions such as oxidative stress and carbon starvation. Due to its specialty characteristics, it is considered as an etiological agent of severe foodborne pathogen infectious diseases – listeriosis. In recent years, the outbreak cases of listeriosis increased gradually, even it is classified as a rare disease but is able to cause high hospitality rate (>90%) and high fatality rate (20-40%) (Jemmi and Stephan, 2006; Meloni et al., 2009). It is ordinarily infected a vulnerable population of "YOPI", such as young, elderly, immunocompromised patients and pregnant women whereby evoke to the incident of miscarriage, stillbirths, septicaemia, meningitis, gastroenteritis, mild flu symptoms, fever and climactically resulting to death (Franciosa et al., 2001; Vazquez-Boland et al., 2001; De Cesare et al., 2007; Silk et al., 2012).

Most listeriosis outbreak cases originated from the transmission of *L. monocytogenes* contaminated food to the host (human body) (WHO, 2018; FDA, 2019). Due to its enduring solid capabilities, this extremophile pathogen is able to grow efficiently on various food surfaces, either on food or in food processing

facilities, even at low temperatures (Colagiorgi et al., 2017). It starts cell proliferation from planktonic into biofilm state for better survival rates when competing with other pathogens under complex communities on various food surfaces. In the form of biofilm, it embedded itself in self-produced matrix of extracellular polymeric material for stronger adhesion to variety of food surfaces in food processing plant such as stainless steel, Teflon, pipe system and etc (Donlan and Costerton, 2002; Colagiorgi et al., 2017). Moreover, the structure of the extracellular polymeric matrix aids as a protection layer to pathogen from antimicrobials and disinfectants invasion. In the usual environment in food processing facilities, biofilms are comprised of multiple bacterial species rather than single-species biofilm (Colagiorgi et al., 2017). It will become resistant to antibiotics and sanitizers than single-species biofilms. This can be one of the challenging tasks for the food handlers in the daily cleaning routine as it is hard to be cleaned with common cleaning methods. Thus, the biofilm production of *L. monocytogenes* becomes a potential vehicle to transport *L. monocytogenes* to food and other food contact surfaces or vice versa and causes severe food spoilage or transmission of invasive diseases, consequently to the occurrence of listeriosis outbreaks.

In Malaysia, there were several prevalence studies reported the presence of *L. monocytogenes* from a variety of food such as meat, beef patties, beef offal, chicken offal, salad, fresh vegetables and ready-to-eat food (Arumugaswamy et al., 1994; Tang et al., 1994; Ponniah et al., 2010; Marian et al., 2012; Wong et al., 2012; Kuan et al., 2013a; Kuan et al., 2013b; Marian et al., 2019). Furthermore, our Ministry of Agriculture and Food Industry has seized the import of *L. monocytogenes* contaminated apples from Bidart Bros in

Bakersfield, California in the year 2015 (Malay Mail, 2015). This shows that food contamination is likely to happen along the way from farm to fork, especially during food processing, storing and distribution to the premises. It also increases the likelihood of the occurrence of cross-contamination of food with soils and wastewater in farms, food contact surfaces in food processing facilities or retail premises environment.

To date, no actual human listeriosis outbreak cases have been reported in Malaysia. Yet, according to the health facts reports from 2008 to 2019, there was an increment in the incidence and mortality rates of food poisoning cases reported in Malaysia from 2016 to 2019. In year 2019, reported food poisoning cases had increased by 24% (Kaur, 2019). However, public awareness of food safety issues in Malaysia is relatively low and not well understood as the main concern is on taste. Moreover, no regulation exists to control the surface of microbial contamination issue. The existence of food regulations that were being implemented in Malaysia was Food Act 1983 and Food Regulation 1985. Both legislations are the backbone of the food safety programme in Malaysia. There are no specific law acts stated in the regulations to control or monitor the surface of microbial contamination matters, especially for *L. monocytogenes*, even though the Ministry of Health established Food Safety and Quality division in 2002. Thus, it is crucial to monitor the food safety level of *L. monocytogenes* in food processing facilities, especially those processing ready-to-eat food products to be consumed directly without any further heating process to eliminate contamination.

1.2 Objectives

Therefore, the general objective of the study was to determine the emergence of *Listeria* spp. and *L. monocytogenes* in food premises and analyze the way that affect the public health and the specific objectives of the study were:

1. To isolate *L. monocytogenes* from food samples and food contact surfaces at food premises,
2. To determine the serotyping group, virulence genes and antibiotic resistance profiles of the isolates,
3. To analyze the adherence, biofilm-forming ability and biofilm formation of ATCC 19112 and environmental isolates of *L. monocytogenes* on various surfaces at different temperatures and further disinfect or sanitize test surfaces with three commercial disinfectants.

CHAPTER 2

LITERATURE REVIEW

2.1 Epidemiology of *Listeria* Species and *Listeria monocytogenes*

Listeria monocytogenes (*L. monocytogenes*) is a widespread bacterium in nature but not well-known foodborne pathogen that capable to cause rare but severe infectious disease, listeriosis. Occurrence of human listeriosis commonly associated with outbreaks or sporadic diseases. In year 1929, Nyfeldt reported the first confirmed human listeriosis case without knowing that the main cause was due to the occurrence of *L. monocytogenes* in food (Nyfeldt, 1929; Bojsen-Møller, 1972). In 1935, Burn found the first listeriosis neonatal case and identified that the infection was caused by *Listerella* (earliest name of *L. monocytogenes*) (Burn, 1935). The first human listeriosis outbreak happened in 1983 caused by the consumption of *Listeria* contaminated foodstuffs (Schlech et al., 1983).

To date, human listeriosis not only infected human invasively but also non-invasively. Initially, listeriosis was known to affect a vulnerable population of "YOPI" (young infant, old, pregnant mother and immunocompromised patients) invasively by showing symptoms such as bacteraemia, meningitis, septicaemia, encephalitis, abortion, stillbirths, endocarditis, central nervous system infection, flu-like symptoms (fever, chills, fatigue, back pain, headache and etc) or even death (Franciosa et al., 2001; Vazquez-Boland et al., 2001; Pagatto and Farber, 2003; De Cesare et al., 2007; Silk et al., 2012). Besides

vulnerable population, more than 70% of listeriosis get involved in persons with liver disease, cancer and diabetes (Goulet et al., 2012). Thus, this group poses a higher risk and particularly prone to invasive infection than healthy person. Yet, this does not mean that healthy person is free from the infection. In the past twenty years, several reports on healthy person infected by non-invasive listeriosis and showing asymptomatic gastrointestinal symptoms of febrile gastroenteritis (Miettienen et al., 1999; Aureli et al., 2000; Hof et al., 2000; Ooi and Lorber, 2005; Jacks et al., 2015). Hence, there is a potential risk of healthy individuals being infected through consumption of *Listeria*-contaminated food.

2.2 *Listeria* Species

Listeria is genus which comprised of a group of Gram-positive, non-spore-forming, facultative anaerobic bacteria with low G+C content. They grow in rod-shaped with the width of 0.5µm and length of 1 to 1.5µm without self-capsule and remain motile at 10 to 25ºC (Vázquez-Boland, 2001; Liu, 2006).

Figure 2.1: Image illustrated the morphology characteristics of *L. monocytogenes*. *L. monocytogenes* grow in rod-shaped and surrounded with flagellums. Adopted from (Bohdan, 2023).

They present in a variety of sources, including nature, food and waste sources such as: soil, water, vegetables, meats, waste water, silage and feces of humans and animals (Farber and Peterkin, 1999; Liu, 2006).

Currently, there are 20 recognized species of *Listeria* genus: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. grayi*, *L. welshimeri*, *L. fleischmannii*, *L. weihenstephanensis*, *L. marthii*, *L. aquatica*, *L. rocourtiae*, *L. floridensis*, *L. booriae*, *L. cornellensis*, *L. newyorkensis*, *L. grandensis*, *L. costaricensis*, *L. riparia*, *L. goaensis* and *L. thailandens* (Orsi and Wiedmann, 2016; Dojiad et al., 2018; Leclercq et al., 2019; Nwaiwu, 2020). Two out of 20 species, *L. monocytogenes* and *L. ivanovii*, are recognized as the most pathogenic species which poses abilities to infect humans and animals (Dojiad et al., 2018). Both species start to invade the host once enter through transmission of contaminated food to the host body. The bacterium will undergo cell replication and spread from cell to cell to proliferate in the host (Guillet et al., 2010).

The difference between both species is that *L. monocytogenes* infects humans and animals, especially ruminants, whereas *L. ivanovii* was recognized only infects ruminants (Guillet et al., 2010). However, there are some reports found that *L. ivanovii* isolated from infected human cases have underlying certain health condition such as immunocompromised, lymphoma, hepatic carcinoma and etc (Guillet et al., 2010; Beye et al., 2016). There are a total of eight human *L. ivanovii* infection cases reported since 1971 (Beye et al., 2016). Even though, *L. ivanovii* had been isolated from environmental and food samples in recent years (Alvarez-Ordóñez et al., 2015; Chen et al., 2017). Yet, the occurrence of the human listeriosis outbreaks due to *L. ivanovii* is rare compared to *L. monocytogenes*.

2.3 *Listeria monocytogenes*

L. monocytogenes is recognized as the causative agent of human listeriosis. It employs food sources as transport vehicles to infect host body with listeriosis. However, the contamination does not mainly due to the food components itself. The occurrence of contamination might start from farm to fork before the consumer purchased it, further to cooking process and it is ready to be served. Along the whole food chain for farm to fork, each of the process has the possibility to get contaminated by *L. monocytogenes* or even cross-contaminate among others. Therefore, *L. monocytogenes* can be found in raw materials to end-products; in environmental sources: farming site processing site and distribution site.

L. monocytogenes commonly found in food samples of raw and processed meats of beef and chicken, beef offal, chicken offal, vegetables, fish, milk, milk products, ready-to-eat (RTE) food products of salads, sushi and others (Arumugaswamy et al., 1994; Hsih and Tsen, 2001; Manfreda et al., 2005; Meloni et al., 2009; Khan et al., 2013; Kuan et al., 2013; Al-Nabulsi et al., 2015a; Kuan et al., 2015; Bucur et al., 2018; Amusan and Sanni, 2019). Other than food samples, *L. monocytogenes* also can be isolated from environmental resources such as soil, waste water from farm and even food processing environments of production line such as stainless-steel working table, rubber material of conveyor belt in packaging machine, flooring tiles and etc (Dowe et al., 1997; Lundén et al., 2000; Garrec, Picard-Bonnaud and Pourcher, 2003; Camargo et al., 2017).

2.4 Pathogenesis of *L. monocytogenes*

As *L. monocytogenes* is being discovered and reported in a variety food and food products in Malaysia as well as other developed countries such as United States, it can lead to the occurrence of listeriosis outbreaks especially towards the "YOPI" populations.

After consuming and coming into contact with *Listeria* contaminated food or food contact surfaces, *L. monocytogenes* will begin its pathogenic journey in the host by entering the digestive tract. *L. monocytogenes* invaded host's body, colonized the intestinal tract, crossed the intestinal barrier, entered the bloodstream, traveled to the target organ, liver (Vazquez-Boland et al., 2001; Radoshevich and Cossart, 2017; Quereda et al., 2021). However, the pathogenesis of *L. monocytogenes* in immunocompromised patients is potentially fatal as *L. monocytogenes* able to travel across the blood-brain and placental barriers and leading to severe neuro-related listeriosis, such as meningitis and sepsis as well as cases of abortion and premature birth (Vazquez-Boland et al., 2001; Radoshevich and Cossart, 2017; Quereda et al., 2021).

Since *L. monocytogenes* has been discovered to be extremely dangerous to the "YOPI" population, research into methods of identifying *L. monocytogenes* and preventing their spread is urgently needed.

2.5 Identification of *L. monocytogenes*

Due to the pathogenicity of *L. monocytogenes* towards human listeriosis outbreak, it is crucial to identify *L. monocytogenes* in food and environmental samples for the sake of public health. Use of relevant identification techniques in epidemiological and clinical studies is necessary to detect and verify the

current food safety status (Khan et al., 2013). Findings from the identification can be used as the evidence to increase the public's awareness towards noxious foodborne bacterium, *L. monocytogenes*.

The key factor of pathogenicity of *L. monocytogenes* is mainly from a novel peptide, named listeriolysin O (LLO) gene. It provides the virulence factor for *L. monocytogenes* to harm the host body. Thus, it was frequently used by various researchers as key marker to identify the occurrence of *L. monocytogenes* from any sources of samples (Leimesiter-Wächter and Chakraborty, 1989; Dumen et al., 2008; Kuan et al., 2013).

2.6 Methods of Isolation and Detection of *L. monocytogenes*

The initial step of detection of *L. monocytogenes* from variety source of samples is utilizing microbiological culture method from isolation to detection via different confirmation methods and classify them accordingly (Gasanov et al., 2005). There are several methods in isolating bacterium by referring to different selective enrichment methods and using particular selective plating method. Once bacterium is isolated from selective plating method, it will be confirmed and classified by biochemical, molecular, rapid method or mixture of methods.

From the previous studies, there are three types of selective enrichment methods based on different standards regulated by distinct government agencies: International Organization of Standards (ISO), Food and Drugs Administration (FDA) and Department of Agriculture in United States (USDA) (Gasanov et al., 2005; Nayak et al., 2015). Based on the regulations of each standard, differential culture media and agar was used for isolation. Among the three methods, ISO 11290 and FDA bacteriological and analytical method (BAM) are the most

widely used method in detection of *L. monocytogenes* from food samples. On the other hand, USDA with Association of Analytical Chemists (AOAC) 993.12 method is the method preferred for dairy products and environmental samples (Gasanov et al., 2005; Nayak et al., 2015).

2.6.1 Biochemical Method for Detection of *L. monocytogenes*

Isolated bacterium can be confirmed by utilizing biochemical methods of Gram-staining, catalase and oxidative test, carbohydrate fermentation, hemolytic activity with Christie, Atkins, Munch-Petersen (CAMP) test and lecithinase activity to detect and differentiate between *Listeria* species and *L. monocytogenes*. All of the *Listeria* species are Gram-positive, rod in shape, catalase-positive, oxidase-negative and ferment carbohydrate with production of acid without gas (Gasanov et al., 2005).

Since *Listeria* species is anaerobic bacteria, it will need to undergo carbohydrate or sugar fermentation when grow in aerobic environment. Under aerobic condition, *Listeria* species will ferment different types of glucose, such as L-rhamnose, D-xylose, D-mannitol or α-methyl-D-mannoside as source of energy to support growth and produce acid as by-product of the carbohydrate fermentation test (Farber and Peterkin, 1999; Gasanov et al., 2005; Jeyaletchumi et al., 2010). *L. monocytogenes* and *L. innocua* able to ferment L-rhamnose, and α-methyl-D-mannoside, whereas *L. ivanovii* and *L. seeligeri* only capable to ferment D-xylose. *L. welshimeri* able to ferment all three types of sugar except D-mannitol and *L. grayi* capable to ferment all three types of sugar except D-xylose (Gasanov et al., 2005).

In addition, each of the *Listeria* species shows different hemolytic activity on sheep or horse blood agar. *L. monocytogenes*, *L. seeligeri* and *L. ivanovii* are *Listeria* species that showed positive hemolytic reaction towards blood agar. Among three *Listeria* species, *L. ivanovii* showed the production of wide, clear, multiple zones of hemolysis. *L. monocytogenes* exhibit a narrow zone of hemolysis without extension too much from the edge of colonies, then followed by *L. seeligeri* with the presence of the narrowest zone of hemolysis out of the three species (Allerberger, 2003; Jeyaletchumi et al., 2010).

With the aid of using β-hemolysin-producing bacteria, *Staphylococcus aureus* (*S. aureus*) and *Rhodococcus equi* (*R. equi*) streaked on the blood agar plate able to improve the differentiation between *L. monocytogenes*, *L. ivanovii* and *L. seeligeri*. This test is named as CAMP test. Hemolytic activity of *L. monocytogenes* and *L. seeligeri* react positively towards *S. aureus* but *L. seeligeri* showed lesser extent compared to *L. monocytogenes*. On the other hand, *L. ivanovii* hemolysis reacts positively towards *R. equi*. However, the reliability of CAMP test is limited due to false positive results (Allerberger, 2003; Gasanov et al., 2005).

Thus, a simple method based on the lecithinase activity by charcoal is introduced in order to differentiate *L. monocytogenes* and *L. ivanovii* without confusion. Plc B phospholipase C also known as lecithinase is used as the pathogenicity marker to differentiate *Listeria* species, especially *L. monocytogenes* and *L. ivanovii*, which are isolated from food samples (Ermolaeva et al., 2003). All non-pathogenic *Listeria* species exhibited negative results in charcoal-supplemented egg yolks agar plates. Only both pathogenic strains of *L. monocytogenes* and *L. ivanovii* indicated the differences between

other *Listeria* species. From the findings, *L. ivanovii* is easily differentiate from *L. monocytogenes* as *L. ivanovii* exhibited strong lecithinase reaction in both with or without the charcoal-supplemented agar plates. For *L. monocytogenes*, it may require the presence of charcoal in the medium in order to show the positive lecithinase activity (Ermolaeva et al., 2003).

2.6.2 Serological Method for Detection of *L. monocytogenes*

Serological method is one of the phenotypes typing methods which using phenotypic markers to detect target organisms. A group of specific surface proteins of somatic "O" and flagellar "H" antigens are the phenotypic markers which react to corresponding monoclonal and polyclonal antibodies that are used to detect *Listeria* species (Gasanov et al., 2005; Liu, 2006). There are a total 15 subtypes of O antigens from I to XV whereas H antigens consists of 4 subtypes from A to D. Based on the determination of the unique combination of O and H antigen for individual *L. monocytogenes* strains were classified into 13 serotypes: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7 (Ermolaeva et al., 2003; Gasanov et al., 2005; Liu, 2006). However, other *Listeria* species shared the similar serotyping antigens and respond accordingly towards monoclonal and polyclonal antibodies. Thus, serological detection method is keen for identification genus of *Listeria* but unable to differentiate among other *Listeria* species. It is applied as the first level of identification tool to confirm and determine the genus of isolated *Listeria* prior for epidemiological studies and work as a tracking mark in contamination studies for environmental specimens (Ermolaeva et al., 2003; Gasanov et al., 2005).

2.6.3 Molecular Method for Detection of *L. monocytogenes*

Due to the advancement of technology in the past few years, a rapid, specific and highly sensitive molecular detection method has evolved into one of the most favourable detection and identification methods for isolated *L. monocytogenes* from varieties of sample. The pioneer and simplest molecular detection method is deoxyribonucleic acid (DNA) hybridization with gene probes. Gene probe is a single strand of specific sequenced nucleic acid. It can be a type of enzyme or radioactive isotope which acts as a label marker to detect the complementary sequence of target organism (*L. monocytogenes*) (Gasanov et al., 2005). Differentiation of *L. monocytogenes* from other *Listeria* species is more precise by using targeting probes to specific gene of virulence factor (Gasanov et al., 2005). For instance, AccuProbe®, a chemiluminescence DNA probe from Gen-Probe, San Diego, CA, USA for rapid confirmation of *L. monocytogenes* on primary isolation agar plate (Ermolaeva et al., 2003). With its convenient and time saving approaches, DNA hybridization is commonly used in food testing as the results is more reliable and specific compared to biochemical method. However, this detection procedure is incapable to screen for clinical samples. Since this technique excludes the nucleic acid amplification step whereby it is limiting the sensitivity of detection of clinical samples which require at least 10^4 copies of target gene per microlitre to obtain reliable result (Liu, 2006). Thus, this technique is being discontinued and replaced by a new evolved molecular method, polymerase chain reaction (PCR).

In order to overcome the limitation of DNA hybridization with gene probes, PCR technique is introduced to the investigators. PCR method involves the nucleic acid amplification step which multiply the number of copies of

certain segment. For instance, clinical samples which comprised of limited number of copies which unable to fulfil the minimal requirement of DNA hybridization with gene probes method for identification of *Listeria* species (Gasanov et al., 2005). However, with the implementation of PCR technique, it can amplify and increase the concentration of target segment by utilizing heat stable DNA polymerase for synthesizing and with a set of primer which flank the initial and end of target segment of DNA based on the virulence gene factor of *L. monocytogenes* (Gasanov et al., 2005; Liu, 2006). The amplified PCR products will be detected by undergoing separation via agarose gel electrophoresis. Hence, PCR method is widely implemented for detection in both research and clinical laboratories even until now for identification of *Listeria* species.

In order to ease the differentiation process between *Listeria* species and *L. monocytogenes*, multiplex polymerase chain reaction (m-PCR) is now well established for detection of *L. monocytogenes* from food and environmental samples. The main differences of m-PCR and PCR is the number of primer sets added in each run of PCR procedure (Gasanov et al., 2005; Liu, 2006). For instance, the most common combination to differentiate *L. monocytogenes* from other *Listeria* species by using 16S rRNA, which provide and hemolysin gene A, *hly*A, which encode for major virulence factor of *L. monocytogenes*, listeriolysin O (LLO) (Yin et al., 2010). Thus, m-PCR is one of the favourable choices of technique for food testing. It is capable to perform low cost and highly sensitive result to commit both purpose of identification and differentiation of *L. monocytogenes* from *Listeria* species or *L. monocytogenes* from other species of bacteria at once.

2.6.4 Rapid Method for Detection of *L. monocytogenes*

Moreover, rapid method detection is another advanced identification method whereby utilizing immunoassays-based concept certified commercial kits by relevant regulatory authorities. It is also known as antibody-based test as genus-specific monoclonal antibodies to *Listeria* species employed in food analysis, especially in food industry (Ermolaeva et al., 2003; Gasanov et al., 2005). With the utilization of immunoassay kits in food industry, it makes analyzes procedures simple and easier without tedious preparation, food investigators able to access results of the analyzes in a shorter time than usual traditional complex culture media procedures (Ermolaeva et al., 2003; Gasanov et al., 2005). Most of the commercial analyzes' kits are invented in accordance with the knowledge towards the techniques of enzyme-linked immunosorbent assay (ELISA) and immuno-capture.

ELISA method is performed in 96 wells microtiter well with immobilized antibodies to capture antigen and combined with a secondary antibody which coupled to an enzyme to detect the captured antigen (Gasanov et al., 2005). This approach is widely used in food testing as food samples comprised of high complexity of different components which can be easier separated and detected though ELISA kit. For instance, TECRA *Listeria* Visual Immuno Assay and *Listeria* Unique® from TECRA International; EiaFoss *Listeria* ELISA kit from Foss Electric A/S and etc (Gasanov et al., 2005). However, commercial kits are mainly not designed for clinical analysis.

Yet, this can be overcome by using the immuno-capture technology. Immuno-capture is a progressive technique which applying specific antibodies coated on magnetic beads or dip sticks to detect *Listeria* species and separate

them from other bacteria species from competing microflora and inhibitory food supply (Gasanov et al., 2005). This method is capable to capture and gather target organisms (*Listeria* species or *L. monocytogenes*) as well as increasing concentration of the target microorganisms in boosting up the sensitivity of the test (Gasanov et al., 2005). For instance, Dynabeads anti-*Listeria* from Dynal Biotech which commonly used for variety food samples (Gasanov et al., 2005). Hence, it is suitable to perform low concentration of target organisms from clinical specimens.

2.6.5 Combination Methods for Detection of *L. monocytogenes*

Other than rapid detection method, combination of any two methods as discussed above is another favourable and commonly used in detection of food and clinical specimens. The widely use combo are: a) culture and molecular method; b) serological and molecular method; and c) immunoassay and molecular method are commonly used in detection of food and clinical specimens.

To date, the most preferable combination method implemented for food, environmental and clinical specimens in laboratories is culture and molecular method, such as direct plating with colony PCR and most probable number (MPN) PCR. Direct plating is the usual traditional culture method with long and complex sample preparation for determination. After long time of incubation, colony of the target microorganisms is used directly as template and run for PCR for confirmation. This method of direct plating with colony is a rapid screening method with a small amount of DNA template from colony to screen out true positives and false positives colonies from large number of colonies grown on agar plates (Bergkessel and Guthrie, 2013). However, this method is only

effective to DNA samples less than 1kb length (Bergkessel and Guthrie, 2013). On the other hand, MPN method is a technique used to estimate number of viable cells in samples in low levels of microorganisms from ≤10-100 MPN g^{-1} with specific identification media. With MPN-PCR method in enumeration viable cell number in samples, it able to exclude the tedious media preparation and long incubation time in traditional culture method. After MPN steps, cells are extracted for PCR analysis to determine the *Listeria* species. The combination of MPN-PCR (MPN with m-PCR) is widely conducted in food and environmental samples (Fredslund et al., 2001; Martin et al., 2004; Chen et al., 2016).

Besides that, combination of serological and molecular method mainly performed in laboratories for food and environmental specimens. It is important to characterize *Listeria* species based on serotypes for epidemiological study and to determine which strains of *L. monocytogenes* is the most pathogenic towards human listeriosis. With implementation of molecular method in serological detection, it helps to save labour cost and time required for analysis. However, there is some limitation in the identification procedures whereby it is difficult to differentiate serotypes between 4a, 4b, and 4c (Liu, 2006). Thus, the identification procedures are focussed on those related to human listeriosis. Among 13 serotypes, only 3 serotypes (1/2a, 1/2b and 4b) are related to human listeriosis. Commonly, it is utilizing m-PCR method with 5 sets of primers of targeting genes of *lmo*0737, *lmo*1118, ORF2819, ORF2110 and *prs* and further group them into 4 different sero-groups (Doumith et al., 2004). Group 1 consists of serotypes of 1/2a and 3a which amplify lmo0737 gene. Group 2 comprised of serotypes of 1/2c and 3c which amplify genes of lmo0737 and lmo1118. Group

3 consists of serotypes of 1/2b, 3b and 7 which only amplify ORF2819 gene. Lastly, group 4 comprised of serotypes of 4b, 4d and 4e which amplify genes of ORF2819 and ORF2110. All *L. monocytogenes* strains have *prs* gene as it is a type of protein (putative phosphoribosyl pyrophosphate synthetase) that target all *Listeria* genus gene marker (Doumith et al., 2004).

Moreover, immunoassays also can be applied together with molecular technique to enhance the effectiveness of the identification process of target microorganisms. Immuno-capture technique applied together with molecular methods to differentiate the target microorganisms. For instance, immunomagnetic separation (IMS) and m-PCR was conducted together to achieve immuno-PCR assay that able to detect *Salmonella* species and *L. monocytogenes* from food samples (Hsih and Tsen, 2001). Hsih and Tsen (2001) had developed a method of IMS-m-PCR whereby using immunomagnetic beads to inhibit food components and increase the concentration of target cells prior for m-PCR for confirmation. This method showed higher sensitivity and purity before confirmation with m-PCR. In the study of Li et al. (2000), IMS-m-PCR and slot blot assay was conducted to identify *Salmonella* species and *L. monocytogenes* for milk samples. Li et al. (2000) reported that the implementation of slot blot after IMS-m-PCR increased the cells detection sensitivity from 10^4 to 10^7 cfu/ml. Thus, this method provides a high accuracy and reliable result in the identification of *Salmonella* and *L. monocytogenes* in milk samples.

2.7 *Listeria* in Food Processing Facilities

In the food industry, *L. monocytogenes* able to adhere to a wide range of food contact surfaces, for instance, polymer materials (plastic), stainless steel, glass and rubber gasket (Barbosa et al., 2013; Colagiorgi et al., 2017; Khelissa et al., 2017). It has been found that *L. monocytogenes* can colonize on food contact surfaces and equipment in the food industry in the long run in the biofilm form. Besides that, it is able to survive under extreme condition such as low temperature (2-4^{o}C), low pH, high salt content, under oxidative stress and carbon starvation (Wu et al., 2015; Colagiorgi et al., 2017; Khelissa et al., 2017). Thus, it is able to persist in most of the surfaces in food processing facilities such as the floor, drains, equipment and others for days, months and even years.

Moreover, improper or inadequate cleaning procedures by food handlers may be another factor that is increasing the risk of the growth and establishment of *L. monocytogenes* in food processing facilities especially for those locations that hard to clean such as the edges of the equipment, blades of meat cutter and suture of the stainless-steel surfaces (Lee et al., 2017; Akabanda et al., 2017). With these factors, it seems able to generate risk to the public health which associated with the food safety concern to the final product produced.

Besides the ineffective cleaning procedures, the incoming raw materials, the farm produces and livestock are also one of the potential sources that introduce *L. monocytogenes* to food processing facilities environment and food chain of a product (from farm to food) (Wiedmann et al., 1996; Castro et al., 2016). Previous studies have found that *L. monocytogenes* has been isolated from the animal-related sources, for instance, animal feed, silage, manure, cattle, water, soil and wastewater. The stated sources may be one of the reservoirs of *L.*

monocytogenes and further transmitted to other sources before reaching to the end process of a food chain (Sauders et al., 2012; Vivant et al., 2013; Linke et al., 2014).

Furthermore, *L. monocytogenes* has been detected from ready-to-eat (RTE) food such as ham, salads and others. All of RTE food mostly undergo a post-processing process in retail facilities, share the same cutting board for all the cutting process, repacking, slicing and wrapping without proper handling method – without gloves (Endrikat et al., 2010; Hoelzer et al, 2011; Chaitiemwong et al., 2014; Scollon et al., 2016). Improper handling method is the main reason for the contamination. In addition, *L. monocytogenes* has been isolated from non-food contact surfaces such as the walk-in cooler shelves in retail facilities. Due to the fluctuation temperature along the way of distribution and commercialization of food products, *L. monocytogenes* able to proliferate at this range of temperature (Endrikat et al., 2010, Hoelzer et al., 2011).

2.8 Survival of *L. monocytogenes* under Stresses Condition

From previous studies, *L. monocytogenes* is being isolated from various types of food and environmental samples and is known to survive, resistant to stress and proliferate under a wide range of extreme environmental conditions (Liu et al., 2005; Ferreira et al., 2014). *L. monocytogenes* able to resist pH condition from 4.5 to 9.5, high salt content up to 40% w/v and remain active at low refrigeration temperature as low as -1^{o}C (Farber and Peterkin, 1991; Liu et al., 2005, Marian et al., 2012; Al-Nabulsi et al., 2015b).

L. monocytogenes has the ability to display adaptive acid tolerance response (ATR) when exposed to acid condition. According to the studies of

Davis et al. (1996) and Chorianopoulos et al., (2011), Scott A (*L. monocytogenes*) remained alive even cells exposed to low pH of 4.5. *L. monocytogenes* that pre-exposed to mild acid condition of pH 4.5 and further exposed to even lower pH of 3.0 showed better survival rate compared to *L. monocytogenes* that without undergoing a pre-exposed acidity condition. This is due to the properties of *L. monocytogenes* in the stage of ATR, ATPase in cell, enzyme is being activated to protect the cell from death through regenerating its expression. At mild acid stress, ATPase of the glutamatic acid decarboxylase (GAD) or arginine (ADI) and agmatine deiminases (AgDI) are involved (Cotter et al., 2000). In mild acid condition, GAD system is being applied in enzymatic reaction by converting extracellular glutamate into aminobutyrate to reduce the proton concentration of intracellular of the cells (Cotter et al., 2001). On the other hand, in extreme acidity condition (≤pH 3), ADI and AgDI are being activated whereby arginine and agmatine from extracellular are imported and further converted into ornithine and putrescine respectively with carbon dioxide, ammonia and ATP (Cotter and Hill, 2003; Chen et al., 2011). Thus, cells that undergo pre-exposed of mild acid can perform better survival than those without.

Moreover, *L. monocytogenes* is capable to survive under high salt condition up to 40% w/v salt environment (Marian et al, 2012). This exhibited that *L. monocytogenes* showed the ability to tolerate the osmotic pressure exerted by the accumulation solutes of glycine, betaine and carnitine between cytoplasm and extracellular environment in the cells. This can reduce the osmotic pressure and water loss via stabilizing structure and functional properties of enzymes (Lippert and Galinski, 1992; Bae et al., 2012). Besides that, *L. monocytogenes* alter the level of specific gene expression aiding the cell growth under high salt

condition. For instance, cspA and cspD genes of cold shock proteins (Csps) will increase in concentration and mediating the chaperone activity to repair the DNA lesions as salt causing DNA breaking (Schmid et al., 2009). However, gene expression associated with carbohydrate reduced and resulting the reduction intake of carbohydrate components when under salt stress (Bae et al., 2012).

Lastly, the most problematic resistance of properties that cause *L. monocytogenes* to be pathogenic infect human and further cause the occurrence of listeriosis outbreak cases is the cryophilic characteristics. It is found to grow and reproduce or proliferate in low temperature of 0 to 4^{o}C and thus *L. monocytogenes* present in refrigerated food products such as milk products and frozen processed food. In low temperature, the metabolic rate of *L. monocytogenes* will be lowered down, composition of membrane will alter, express cold shock proteins and intake cryoprotective compounds (Neunlist et al., 2005; Cordero et al., 2016). Alteration of membrane composition by the increment of unsaturated fatty acid concentration that assists retention of fluidity cytoplasmic content in the membrane (Beales, 2004). In addition, *L. monocytogenes* able to express highly rigid structure of gene proteins of Csp A, Csp B and Csp D under low temperature whereby two types of Csps protein (Csp A and Csp D) also response to high salt stress (Schmid et al., 2009). However, they function differently in salt stress environment. It plays role as molecular chaperones to stabilize and facilitate nucleic acid to replicate, transcript and translate to prevent degradation (Lee et al., 2012; Barria et al., 2013).

2.9 Antibiotics Resistance towards *L. monocytogenes*

L. monocytogenes is not only resisted in extreme condition to growth. It is also found to be resistant towards some types of antibiotics. Clinically, antibiotics are being used to treat *L. monocytogenes* infected patients. They are variety of clinical antibiotics being prescribed to the patients based on their health condition. Yet, not all types of antibiotics are susceptible to *L. monocytogenes*. Studies found that most strains of *L. monocytogenes* resist to antibiotics of tetracycline and fluoroquinolone (Granier et al., 2011; Morvan et al., 2020). There are two mechanisms of *L. monocytogenes* involved in resistance to tetracycline via efflux by proton antiporters: conferring to tetracycline only [*tet*(L)] or resistance to both minocycline and tetracycline [*tet*(S) and *tet*(M)]. Morvan et al. (2020) found that 41% of strains exhibited *tet*(M) and this able confirmed that tetracycline resistance in *L. monocytogenes* is mainly mediated by conjugation mechanism but not via efflux mechanism as *tet*(K) and *tet*(L) determinants was absent.

On the other hand, resistance to fluoroquinolone in *L. monocytogenes* is performed via conjugation of efflux pump. Morvan et al. (2020) revealed that in the presence of reserpine resistance of antibiotic of ciprofloxacin (a type of antibiotic under group of fluoroquinolones) detected from all tested strain to activate the efflux pump via overexpression of *Ide* gene. However, the overexpression of *Ide* gene is not the only factor that affect the resistance of ciprofloxacin in *L. monocytogenes*. Resistance of ciprofloxacin in *L. monocytogenes* can be affected by the utilization of mutated efflux protein regulator of *fep*A as usage of different efflux protein response differently in

adaptation of bacteria to the antibiotic (Romanova et al., 2006; Olaimat et al., 2018; Wilson et al., 2018).

2.10 Biofilm and Biofilm Formation

Biofilm is a substance which consists of an aggregation of viable and non-viable microorganisms embedded in a self-produced matrix-enclosed extracellular polymeric substance that adherent to each other or on a surface. It occurs broadly in nature and is a notable fact in many industrial activities, especially in food industrial activities. In the production line of the food product, microorganisms have a high tendency to grow and adhere to the surfaces and aggregate into a complex structure, known as a biofilm. When bacteria grow into biofilm, they will gain some advantages of getting persistence towards the extreme environmental conditions, resist against antimicrobial agents and hardly to be removed or destroyed (Khelissa et al., 2017).

The main reason of getting persistence is that in a complex structure, extracellular polymeric substances (EPS) consist of polysaccharides, proteins, phospholipids, nucleic acids, teichoic acids and other polymeric substances and 85 to 95% of water. EPS aids as a protection layer towards bacteria cells that inhabitant in biofilm structure by preventing the access of biocides, antimicrobial agent and toxins that able to destroy or remove them from the living space. With these abilities, it is able to grow persistently on the adhered surfaces and become a potential source of contamination in food products in the food industry (Rodríguez-López et al., 2018)

Biofilm formation comprises of four stages: initial attachment, microcolony, EPS production and maturation. Attachment of bacterial cells and

substratum is triggered when the distance between both substances approach 50m as Van der Waals interaction will be involved in the attraction. When the distance becomes closer and closer between 10 to 20mm, non-covalent forces: electrostatic, acid-base and hydrophobic forces will be involved to build for stronger adhesion process. In this stage, the adhesion process still known as a reversible attachment which can easily to be removed by mild shearing force. In the between of 5 to 30s, the reversible attachment will be promoted into an irreversible attachment (Khelissa et al., 2017; Rodríguez-López et al., 2018)

Once the irreversible attachment is established, bacterial cells will be synthesizing the extracellular polymers. The EPS will be accumulated within hours, and the bacteria cells will be enclosed and trapped inside the protection layer EPS and further formed into a mature biofilm. Once the biofilm formation reached the last stage, with a matured EPS matrix, they will become difficult to be removed or destructed with the traditional cleaning methods. They will require some applications of strong shear force: scrubbing or scrapping, applications of strong detergents, sanitizers or heat to loosen the attachment and thus destroy the biofilm. However, it was not that easy to eliminate a mature biofilm; therefore, the most effective way is to reduce the occurrence of biofilm formation by understanding them in details in order to prevent the formation from the initial stage (Khelissa et al., 2017).

2.10.1 Biofilm formation by *L. monocytogenes*

The biofilm that formed by *L. monocytogenes* is common to be found in the food industry facilities. Recently most of the foodborne outbreak cases associated with *Listeria* were mostly detected from the food industry site, because of *L.*

monocytogenes capable of attaching to the various type of surfaces and further developed into biofilms. Therefore, recent studies researchers have been focussed on the biofilm formation by *L. monocytogenes*. It was varying among the serotype, lineage, origin of isolation, intrinsic and extrinsic factors: nutrient levels and temperature. Biofilm matrix produced by *L. monocytogenes* in the irreversible attachment also poses as an important component in biofilm formation of *L. monocytogenes*. All of these factors may be responsible for influencing the biofilm development that produced by *L. monocytogenes.*

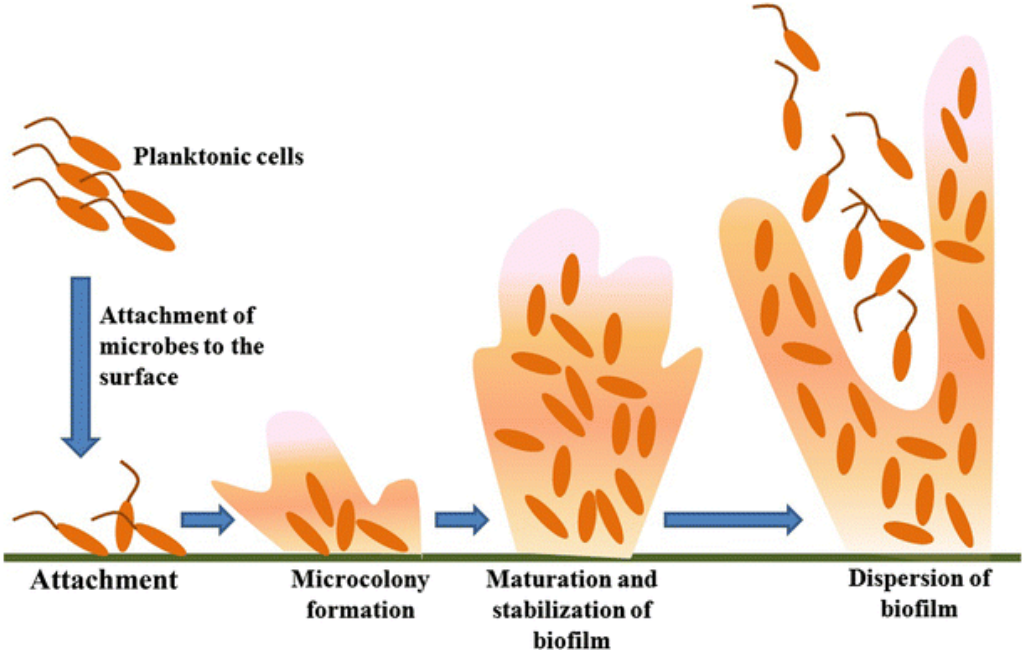


Figure 2.2: Image illustrated the development of biofilm formation of *L. monocytogenes*. Planktonic cells of *L. monocytogenes* attach on surface material, grow into microcolony and continue to grow into mature forms (biofilm) before dispersion of biofilm and free the cells. Adapted from (Gupta, et al., 2023).

2.10.2 Serotype, Lineage and Origin of Isolates of *L. monocytogenes*

From the previous studies, researchers found lineage I strains showed better biofilm forming ability compared to lineage II strains (Djordjevic et al., 2002). However, other researchers found that serotype of 1/2a and 1/2c strains (lineage II) produced more biofilms than 4b serotype strains (lineage I) (Borucki et al.,

2003; Harvey et al., 2007; Pan et al., 2010; Nilsson et al., 2011; Combrouse et al., 2013). Hence, there is still a controversial between the correlation of serotype or lineage when describing the biofilm forming ability of *L. monocytogenes*. According to Nilsson et al. (2011) the origin of the bacterial isolates might have the possibilities to affect the ability of formation of biofilm. However, this finding was then excluded as Kadam et al. (2013) had found that there is no significant difference between the strains in their forming abilities based on the factor of the strain's origin. In the study by Kadam et al. (2013), they found that the extrinsic factors, temperature plays a vital role in differentiating the strain's biofilm forming abilities.

2.10.3 Intrinsic and Extrinsic Factors Affecting Biofilm Forming Capacity

2.10.3.1 Intrinsic Factor

Intrinsic and extrinsic factors also act as the crucial factors that are influencing the biofilm forming abilities of *L. monocytogenes*. Intrinsic factors such as nutrient level, bacterial surface hydrophobicity and surface charge in bacterial adhesion. According to the study of Harvey et al. (2007), the team found that the different growth medium that used in forming biofilm showed significant results. *L. monocytogenes* formed an enormous amount of biofilm in Modified Welshimer's broth (MWB) when compared to Tryptone Soy Broth (TSB) and diluted 15-fold of TSB. It can be concluded that nutrient available in the medium may influence the biofilm forming capacity.

Due to the limited biofilm forming accessories in *L. monocytogenes*, the cell surface of hydrophobicity for adherence and biofilm formation act as an essential factor to affect adherence ability to the surface. The strain of *L.*

monocytogenes with higher hydrophobicity index (HI) classified as robust biofilm former when compared to low HI which is a weak biofilm former (Dojiad et al., 2015). Nonetheless, the HI will depend on many other parameters such as pH, the ionic strength of growth medium and bacterial species (Khelissa et al., 2017).

Surface charge is also another crucial physical factor for bacterial adhesion. In general, bacteria will have a net charge of negative charge, but it will vary among bacteria species, bacteria age and surface structure, growth medium, pH and ionic strength of growth medium. Nevertheless, they have not been well studied on *L. monocytogenes*. Researchers revealed that the adhesion rate of *Staphylococcus epidermis* is directly correlated to the negative surface charge, but the adhesiveness of *E. coli* is inversely proportional to the surface electro-negativity (Khelissa et al., 2017).

2.10.3.2 Extrinsic Factors

On the other hand, the extrinsic factors that involved in determining the biofilm forming ability of *L. monocytogenes* are temperature, pH, osmotic level and environmental stresses. Among the listed factors, previous studies proved that temperature is the essential factor that favouring the biofilm formation of *L. monocytogenes* (Midelet et al., 2006). From the previous studies, most of the researchers focussed their studies on the temperature at a higher temperature around 37ºC, as this is the *Listeria*'s optimum growth temperature whereas at 25ºC is where bacterial cells able to present their flagella for motility purpose (Colagiorgi et al., 2017).

Production of EPS of *L. monocytogenes* was higher at both temperature of 22ºC and 37ºC when compared to 4ºC and 12ºC (Bonaventure et al., 2008; Tomičić et al., 2016). However, from this study, it showed that biofilm of *L. monocytogenes* able to form biofilm at low temperature as 4ºC and 12ºC. In addition, in the study of Chavant et al. (2002), they observed that the ability of *L. monocytogenes* to colonize on the surface material of polytetrafluoroethylene (PTFE) was at 37ºC instead of 4ºC.

Moreover, the researcher also observed biofilm forming ability of *L. monocytogenes* at a refrigeration temperature of 4ºC. Biofilm formation on stainless steel surface was found higher compared to glass and polystyrene surfaces at 4ºC (Norwood and Gilmour, 2001; Bonsagila et al., 2014). Based on this study, a biofilm of *L. monocytogenes* able to regulate specific gene that is unable to be expressed at higher temperature process. Thus, *L. monocytogenes* capable of forming into biofilm at low temperature and further increasing the food contamination rate along the storage and transportation period (Piercey et al., 2016).

On the other hand, researchers have reported that there is a relationship between pH and osmotic level and the ability to produce biofilm (Djordjevic et al., 2002). Environmental stresses such as carbon starvation and nutrient starvation also play a dominant role in affecting the attachment and development of biofilm (Folsom et al., 2006; Begley et al., 2009). According to Barbosa et al. (2013), the ability to produce biofilm at 37ºC was reduced after the exposure of acidic sublethal stress condition. However, this was not workable on the osmotic exposure at 37ºC. Both acidic and osmotic sublethal stress condition also unable to reduce or increase the biofilm forming ability of *L. monocytogenes* at 4ºC

(Barbosa et al., 2013). Nonetheless, *L. monocytogenes* which has been isolated from the environment of the cheese-making industry, they showed the response towards acid and salt stress (Adrião et al., 2008). Thus, the adaptation of *L. monocytogenes* towards environmental stress may aid as an enhancer or diminisher to the ability of biofilm formers.

2.10.4 Factors Affecting Biofilm Forming Abilities of *L. monocytogenes*

Besides the factors that were being listed as above, there are few factors that necessary to be understood in order to have a better understanding towards biofilm forming abilities of *L. monocytogenes* and thus, generate an effective control strategy to eliminate the growth of biofilm in the food industry. The parameters that capable of controlling the forming abilities of *L. monocytogenes* are the chemical properties of the food contact surfaces, the surface energy and its hydrophobicity degree and surface topography and roughness.

2.10.5 Chemical Properties of Food Contact Surfaces

The ability of bacteria attach to an abiotic surface and form into biofilm depends on the chemistry of solid surface. The chemical properties of a contact surfaces are correlating to the properties of the surface charges and hydrophobicity degree of the attached surface (Chmielewski and Frank, 2003; Khelissa et al., 2017). Different type of material surface will contain different functional groups as this indirectly influence the adhesiveness of the surface towards the bacterial cells (Khelissa et al., 2017). For instance, a surface with a coating, the chemistry properties will be altered as the coating might react differently towards the bacteria cells. According to James and Jayakrishnan (2003) and Kadam et al.

(2013) with a layer of thiocyanate or nisin coating on the PVC material, the chemistry of the material surface is modified, where it inhibits the bacterial adhesion by decreasing the hydrophilicity of the native PVC.

2.10.6 Properties of Surface Energy and Hydrophobicity Degree

In the previous parameter stated that, with the addition of a coating on the surface material able to alter the hydrophilicity characteristics of the material. This is due to the reason of surface tension (surface energy) of the contact surface is being modified too. Additionally, the coating has reduced the free surface energy. Once the free surface energy is reduced, the adhesion rate of bacterial cells to the surface will be inhibited. On the other hand, attachment of bacterial cells will be maximized upon high free surface energy of a surface (Blackman and Frank, 1996; Hyde et al., 1997; Mafu et al., 1990).

According to Mafu et al. (1990) and Snide and Carballo (2000), they reported that surfaces with high free energy are surfaces with hydrophilic properties such as stainless steel and glass materials. These surfaces have higher possibilities of getting greater bacteria attachment and biofilm formation than those hydrophobic surfaces such as Teflon, nylon, rubber and fluorinated polymer (Mafu et al., 1990; Hyde et al., 1997; Snide and Carballo, 2000). Smoot and Pierson (1998) and Snide and Carballo (2000), they demonstrated that the initial attachment of *L. monocytogenes* on stainless steel surfaces was more rapid than to rubber.

2.10.7 Properties of Surface Topography and Roughness

In the food industry, stainless steel, rubber, Teflon, nylon and polymer material are the most common materials used for utensils, equipment, equipment parts and gasket. Most of them are abraded and repeated use for several times until they are worn off (Holah and Thorne, 1990). Due to the repetition of usage, they have created a harbourage condition for bacterial growth and thus increasing the ability to entrap bacteria further generate a potential root of food contamination (Chmielewski and Frank, 2003).

In previous studies, most of them focussed on the surface topography, and they revealed that uneven and rough surface able to enhance the bacterial adhesion rate and biofilm development whereas even and smooth surface showed the reduction of adhesion rate (Khelissa et al., 2017). This is mainly due to there will be a greater contact surface area provided on a rough surface when compared to a smooth surface. The greater the contact surface area, the higher the protection rate towards the adhered bacteria. Bacterial cell would prefer to colonize and survive under a shelter against antimicrobial agents and cleansing agents (Khelissa et al., 2017).

According to Faille and Carpentier (2009), the porosity of the surface material showed significant response towards the ability of bacterial attachment and the biofilm development. Research revealed that porous material has a higher infection rate compared to a dense material. With this, it can conclude that bacterial cells prefer to adhere and colonize on the porous and grooved surface due to its larger contact surface area compared to a flat and dense material (Faille and Carpentier, 2009; Khelissa et al., 2017).

2.11 Control and Elimination Strategies towards Biofilm by *L. monocytogenes*

In recent years, due to the increasing listeriosis outbreak cases being remarkable, the tolerance to disinfectants in *L. monocytogenes* has been a topic of concern in the food industry and public health. Some reasons are causing the cleaning methods to become ineffective leading to severe and life-threatening foodborne outbreak cases.

Some of the reasons may be due to the interference with the organic matters especially when in the presence of a high concentration of bacteria. Anthropologic factors such as improper rinsing method and low dosage of disinfectants also responsible for the efficacy of the disinfectants commonly used in the food industry. In addition, *L. monocytogenes* able to tolerate to some disinfectants as some of them may contribute to the persistence of the *L. monocytogenes* in the food industry especially after the exposure to the absolute concentration (Rodríguez-López et al., 2018).

In this session, the working mechanisms of a few cleaning methods will be discussed. The cleaning methods consists of chemical methods, biological methods and other methods in order to control the biofilm formation of *L. monocytogenes* in the food processing facilities.

2.11.1 Effect of Chemical Agents (Chemical Method) on Biofilm by *L. monocytogenes*

Biofilms can be controlled by using chemical agents such as biocides, antibiotics and ion coatings. Previous studies showed that the most common chemical agents that are being used in the food industry for removing the biofilm of *L.*

monocytogenes are disinfectants that mainly consist of under classification of quaternary ammonium compounds, chlorine-based compounds and acid-based compounds.

2.11.1.1 Quaternary Ammonium Compounds

One of the most common biocides used in the food industry for a disinfectant purpose is quaternary ammonium compounds (QACs). It is useful in eliminating bacteria, algae, fungi, spores, mycobacteria and viruses even with low concentration (Gerba, 2015). The main reason of using it as one of the conventional agents in the food industry is that it is surface active agents with low toxicity and mildly affected by organic matter (Rodríguez-López et al., 2018). The working principle of QACs is that it acts actively towards membrane of bacterial cells, disrupt the phospholipid bilayer, cause the cell to burst by leaking the cellular content and eventually cause death (Gerba, 2015).

However, QACs can easily be influenced by environment condition, and genetic background of each of the target strains as it is diverse. Once QACs is overuse or misuse, it may enhance certain genetic elements that can be horizontal transferred. With this, *L. monocytogenes* may have the chance of gaining a new development of new tolerances ability, and thus enhance the biofilm formation of *L. monocytogenes* in the food industry (Dutta et al., 2013; Shapiro 2015).

Moreover, when biofilm of *L. monocytogenes* is exposed to QACs, the membrane fluidity of the cell will be altered as it increases the tolerance to QACs. The main reason for the alteration is that the proportion of iso-C15 and anteiso-C15 branched chain fatty acids is decreased along with a significant increase in the amount of the saturated fatty acids. As a result, the hydrophobicity of the

surface membrane of the biofilm complex will increase gradually and thus promoting stronger adhesion ability to surfaces (Miladi et al., 2013; Muller et al., 2014). Nonetheless, Sinde and Carballo (2000) reported that with QACs washing method, it able to remove the attachment of *L. monocytogenes* on stainless steel, rubber and PTFE surfaces by 246.3 cfu/mm^2, 598.6 cfu/mm^2 and 379.7 cfu/mm^2 respectively.

2.11.1.2 Chlorine-Based Compounds

Besides biocides, chlorine-based compounds antimicrobial agent such as sodium hypochlorite, chlorine dioxide gas and aqueous chlorine dioxide have been proven to be effective against *L. monocytogenes* (Vaid et al., 2010). the price of the chlorine-based compounds is low and effective in eliminating bacteria, fungi and algae. The working principle behind chlorine-based compounds is that they will penetrate directly into the cell forming N-chloro groups and further interfere the cellular metabolism with its fast-oxidizing nature (Wei et al., 1985).

For instance, 3mg/ml of chlorine dioxide with the 90s of exposure time, *L. monocytogenes* in brine chilling solutions reduced about 4 $\log_{10}$ cfu/ml (Valderrama et al., 2009). However, continuous treats the contaminated samples with an increasing concentration of sodium hypochlorite; it can generate higher MIC values against this disinfectant (Lundén et al., 2003). This showed that there is a decreased activity of the sanitization with sodium hypochlorite. According to Valderrama et al. (2009), they reported that the reduction of the effectiveness was due to the influences by the extrinsic factor, the interaction with external elements such as organic matters.

Adherence surface may affect the effectiveness of the removal of biofilm in *L. monocytogenes*. Bremer et al. (2002) had reported that *L. monocytogenes* that grown on stainless steel coupons could be effectively removed compared to the *L. monocytogenes* that grown on polyvinyl chloride surfaces. Moreover, in the studies in Pan et al. (2006) also revealed that with chlorine control method biofilm on the stainless-steel surface was less resistance compared to those grown on the Teflon surfaces. Some researchers found that the efficacy will be decreased when biofilm pre-treated with peroxide-based products, then further treated with chlorine as the biofilm of *L. monocytogenes* has to tolerate to the peroxide-based products, which also act as an oxidizing agent to *L. monocytogenes* (Pan et al., 2006).

2.11.1.3 Acid-Based Compounds

Besides quaternary ammonium compounds and chlorine-based compounds, acid is one of the other choices of disinfectants that able to eliminate the bacterial cells. Acid compounds are considered as strong oxidizers that can interfere with the phospholipid bilayer of cells and damage the cytosolic material and eventually caused death to the cells, is irreversible damage to the cells (Denyer et al., 1998; Maillard, 2002).

Cotter and Hill (2003) reported that the efficacy of the acid compound to *L. monocytogenes* showed different mechanisms as a typical bacterial cell that has the ability to adapt low pH environment by naturally or induced artificially. Due to this reason, *L. monocytogenes* able to survive and protect itself in such low pH condition. Not only that, it is also capable of resisting the extreme and becoming more virulent in low pH environment and thus, it has the ability to

survive and proliferate in the gastrointestinal tract and macrophage phagosome (Cotter and Hill, 2003).

Moreover, acid compounds showed similar working principle as QACs in *L. monocytogenes*, in the exposure of acidic condition, the composition of the cytoplasmic membrane and ratio of iso and anteiso branched-chain fatty acids will be modified accordingly. While in the biofilm of *L. monocytogenes*, the acid-tolerance ability seems like strain dependent. In the exposure of peracetic acid on three different strains showed different resistance threshold level. In addition, the resistance threshold value towards peracetic acid to *L. monocytogenes* varies against the age of the biofilm and the type of surfaces grown on (Ibusquiza et al., 2011).

The overall acid tolerance rate of *L. monocytogenes* is also influenced by the continuously exposed to acid compounds as it will indirectly promoting the adherence ability as it will be more virulent along the way of exposure (Stopforth et al., 2002; Cataldo et al., 2007; Chorianopoulos et al., 2011). In order to eliminate or control the biofilm development of *L. monocytogenes*, additional accompanying strains of lactic acid bacteria, not only act as a protective substance to *L. monocytogenes* in a mixed species biofilm but it also able to increase its tolerance rate to acid compounds disinfectants (Van der Veen and Abee, 2011).

2.11.2 Effect of Bio-sanitation (Biological Method) on Biofilm by *L. monocytogenes*

Besides chemical method, biological method also is encouraged to be implemented in the food industry, as the food handlers have not well understood

this practice for controlling the biofilm formation of *L. monocytogenes* and it is able to solve some of the issues that had been risen in the cleaning methods with chemical compounds. Bio-sanitization is a biological method that is using the aid of microbiota such as lactic acid bacteria and bacteriocins to control the development of biofilms. It is cost effective, eco-friendly and low toxicity to human.

With the high nutrient contents in the food processing environment, there will be much microbiota found in the food processing facilities. Detected microbiota may be act as an enhancer or diminisher to enhance or inhibit the colonisation process of *L. monocytogenes* on the food contact surfaces in the food industry. Moreover, improper usage of microbiome as an antilisterial species to reduce the presence of *L. monocytogenes* may cause undesired food safety issues which may promote to food spoilage and food contamination issues (Fox et al., 2014).

However, there are several types of lactic acid bacteria (LAB) and bacteriocins are known to be highly active against Gram-positive bacteria such as *L. monocytogenes*. Moreover, the presence of some antilisterial structural bacteriocins genes in LAB have been found and reported by Fontana et al., 2015. According to the studies of Zhao et al. (2004 and 2013), they had examined that LAB and its produced bacteriocins able to act as a tool to control biofilms in the food industry. They are able to prevent and showed significant effect towards pre-formed biofilm formation of *L. monocytogenes* and able to extend the efficacy of the treatments up to 3 weeks.

Furthermore, according to the studies by Bower et al. (2005), they discovered that with the use of nisin, bacteriocins coated on the silica surfaces,

it able to show effectiveness to inhibit the growth of *L. monocytogenes*. In addition, once increases the concentration of the coated nisin to the adhered surface, the attached cells were found to be death.

2.11.3 Effect of Other Control Methods on Biofilm

Instead of using chemical and biological methods as the biocontrol methods towards the development of the biofilm in the food processing environment, there are some other methods were being proposed by the researchers, such as the bacteriophage control method and physical methods. The bacteriophage control method will be other control methods that might be not similar working principles as other control methods. Bacteriophage works through the aid of a group of viruses that able to infect bacteria naturally without toxin-production, highly specific and feasible in controlling biofilm formation.

The working principles of bacteriophage are that it can work on itself or coexist with a host. It will be attached to the target bacterial cells and by inserting itself into the bacterial genome and destroy them. Studies had found that bacteriophage capable of destroying or removing *L.* growing biofilm colonies on silicon catheter, a type of medical instruments (Curtin and Donlan, 2006). In addition, according to Augustin et al. (2004), a bacteriophage of ATCC 23074-B1 helps in inactivation the growth of *L. monocytogenes*.

Besides using bacteriophage to help in removal biofilm formation in food processing facilities, instrumental devices such as ultrasound treatment, high pulsed electrical fields and super high magnetic fields also are other methods of controlling the biofilm formation in the food industry site. This method is known as a physical method.

According to the studies of Kabwanga et al. (2018), they demonstrated that with a low current of 200 to 400mA, planktonic cells of Gram-positive and Gram-negative able to be destroyed by transmitting the current through silver, carbon and platinum electrodes. Moreover, with a layer of coated with Nano-plasma trimethyl silane (TMS) on stainless steel surface contribute to inhibiting the growth of biofilm of *S. epidermis*. However, there are no related studies had shown the application of physical methods in controlling the biofilm formation of *L. monocytogenes* in food processing facilities.

CHAPTER 3

METHODOLOGY

3.1 The Overview of the Research Flow

An overview of the research workflow is summarised in **Figure 3.1**.

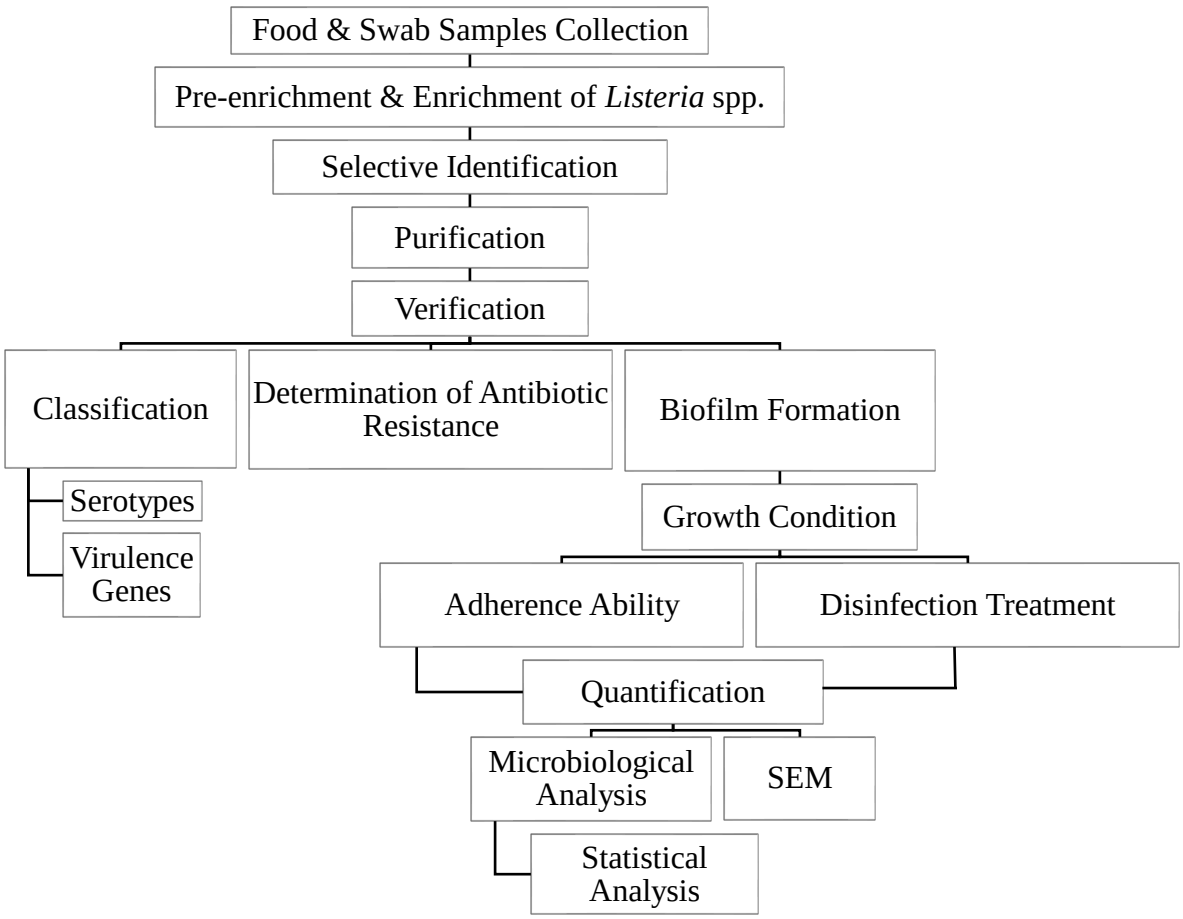


Figure 3.1: An overview of the research workflow.

3.2 Collection of Samples

A total number of 322 samples (170 food samples and 152 food contact surface samples) were randomly collected from six food premises, two processing plants, four hypermarkets, a wet market and a night market in Perak, Malaysia from August 2018 to 2019. The collected samples comprised of food samples (raw, minimally processed, processed and ready-to-eat food) and swab samples from direct and indirect food contact surfaces. The samples collected are summarized in **Table 3.1**.

Table 3.1: Number of samples (shown in bracket) collected in this study.

Source/Total	Samples Collected
Food (170)	
Raw Food (38)	Vegetables (23) Fresh meat and seafood (10) Bean sprouts (5)
Minimally Processed Food (33)	Minced meats (2) Pre-cut meats (5) Pre-cut fishes (6) Pre-cut vegetables (18) Bean curds (2)
Processed Food (6)	Quick-frozen meat products (4) Vacuum-packed meat products (2)
Ready-to-eat (RTE) Food (93)	Sandwiches (13) Salads (10) Fruits (12) Sushi rolls (6) Desserts (25) Cooked food (27)
Food Contact Surfaces (152)	
Direct Food Contact Surface (93)	Working benches for food processing and food preparation (25) Food processing machines (19) Cutting boards (7) Conveyor belts (9) Food preparation tools (mixing bowl, knife, scooper and labelling tools) (22) Surfaces of food product (11)

Table

Indirect Food Contact Surface (59)	Trolleys (2) Display racks (9) Rack stands (6) Racks for intermediate storage (9) Pallets (5) Walls of cold storage environment (4) Cleaning equipment (wiping clothes) (5)

3.2.1 Food Samples

Food samples were purchased freshly from the hypermarkets, wet market and night market. Different categories of food sample were picked randomly from different stalls of each sampling site. Raw, minimally processed and processed foods were kept separately using individual bags in cold storage boxes, whereas ready-to-eat foods were kept in room temperature while transporting back to the laboratory for analysis.

3.2.2 Swab Samples of Food Contact Surfaces

Swab samples from food contact surfaces were collected according to the protocol described by Public Health England (2017) with slight modifications. Briefly, individually packed sterilized cotton swab was pre-moistened with 0.85% (w/v) of saline solution (Merck, Germany). Then, sterilized swab template of size of 10 × 10 cm was placed on the tested area and was swabbed from left to right, up to down and at diagonal sides for 30 s (**Figure 3.2)**. Rotation of the swab was performed during the collection process. The swabbed cotton was placed into a labelled tube containing 10mL of 0.1% (w/v) peptone water (LAB M, United Kingdom). Collected samples were stored in a cold storage box during transportation to the laboratory. The samples isolation was then carried out within 24 hrs.

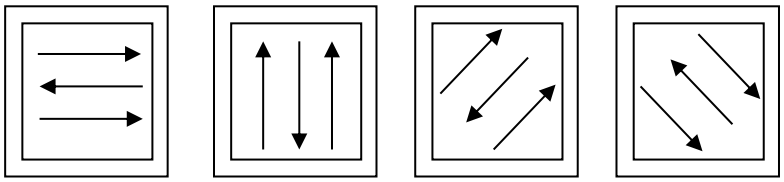


Figure 3.2: Swabbing techniques for collection of swab samples. Direction of swabbing are from left to right, up to down, and on both diagonal sides. Adopted from (Public Health England, 2017).

3.3 Pre-enrichment and Enrichment of *Listeria*

The procedure for detection and isolation of collected samples was performed based on the procedure described by Kuan et al. (2013a) and Kuan et al. (2013b) with slight modifications.

3.3.1 Food Samples

A 25 g of food sample (from section **3.2.1**) was placed in a sterile stomacher bag and homogenized with 225 mL of Listeria Enrichment Broth (LEB) (Merck, Germany) for 2 min using stomacher machine of BagMixer® 400P (Interscience, France). The suspension of 250 mL was then incubated for 4 h at 30ºC, before further enriched with selective supplements agents of acriflavin, 10 mg/L, sodium nalidixate, 40 mg/L, cycloheximide and 50 mg/L (Merck, Germany). Enrichment was then performed for 44 h, incubated at 30ºC.

3.3.2 Swab Samples of Food Contact Surfaces

Collected swab samples of food contact surfaces (from section **3.2.2**) that had immersed in 0.1% (w/v) peptone water was vortexed for 3 min. A 1 mL portion of the suspension was then transferred and homogenized in 9 mL of LEB and

incubated for 4 h at 30°C before enrichment supplements were added. The samples were further incubated for 44 h at 30°C.

3.4 Purification of *Listeria*

After 48 h of incubation, 0.1 mL of broth from all samples was spread plated on Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) agar (Oxoid, UK) and incubated for 48 h at 30°C. Five presumptive colonies with morphology of grey-green colonies with black centre was picked from each PALCAM agar plate and then sub-cultured onto Tryptic Soy Agar (TSA) (Merck, Germany). TSA agar was incubated for 24 h at 30°C.

3.5 DNA Extraction

The boiled cell method was used to extract the DNA of the presumptive colonies from TSA plates, as described by Kuan et al. (2017). Briefly, one loopful of culture was scrapped from the TSA plate and resuspended in 200 µL of sterile distilled water. Suspension was vortexed prior to the boiling step at 100°C for 10 min. It was cooled at −20°C for 10 min before it was centrifuged at 13,400 × g for 3 min. The supernatant was subjected to duplex polymerase chain reaction (d-PCR) to identify and verify *Listeria* species and *L. monocytogenes* strains.

3.6 Duplex Polymerase Chain Reaction (d-PCR)

d-PCR was carried out using two set of primer pairs: a) LI1 and U1, sequences as LI1-5’ CTC CAT AAA CGT GAT CCT 3’ and U1-5’ CAG CMG CCG CGG TAA TWC 3’; b) LM1 and LM2, sequences as LM1-5’ CCT AAG ACG CCA ATC GAA 3’ and LM2-5’ AAG CGC TTG CAA CTG CTC 3’. The first pair was

a genus-specific primer for *Listeria* species which amplified at 16S rRNA gene and generate a size of 938 bp amplicon, whereas the second pair was a species-specific primer for *L. monocytogenes* amplified at hemolysin A (*hly*A) gene and generate a size of 702 bp amplicon (**Table 3.2**). Both primer pairs were synthesized by Apical Scientific Sdn. Bhd.

The reaction mixture of 25 µL containing 5 µL of 5× PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 0.2 µL of 10 mM deoxynucleoside triphosphates (dNTPs) mix, 0.3 µL of 1.5U Taq DNA Polymerase, 0.5 µL of 0.4 µM LI1 primer, 0.5 µL of 0.4 µM U1 primer, 0.5 µL of 0.2 µM LM1 primer, 0.5 µL of 0.2 µM LM2 primer, 14.0 µL of sterile distilled water and 2.0 µL of DNA template (supernatant from the extraction of DNA in section **3.5**) was prepared (**Table 3.2**). All reagents used in the PCR amplification were obtained from Promega (Research Instruments, USA). *L. monocytogenes* ATCC 19112 was used as a positive control for each PCR assay.

d-PCR conditions used was as such: initial denaturation at 94ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 53ºC for 1 min and extension at 72ºC for 2 min, followed by a final extension step at 72ºC for 7 min. The thermal cycling reactions were performed using Thermal Cycler (Matrioux, Malaysia). PCR products were then subjected to 1.5% agarose gel electrophoresis in 0.5× of Tris-Borate-EDTA (TBE) buffer at 100V for 45 min. The gel was then stained with 3× gel red (Biotium, US) and visualied under gel imager (Bio-rad, USA). A 100 bp Plus DNA ladder (Vivantis Technologies, Malaysia) was used as a DNA marker to estimate the size of amplified PCR products.

Table 3.2: The d-PCR mixtures with primers' sequences used to verify presumptive isolates of *L. monocytogenes*.

Reagent	Amount (µL)
5× PCR Buffer	5.0
25 mM $MgCl_2$	1.5
10 mM Deocynucleoside Triphosphate Mix	0.2
1.5U Taq Polymerase	0.3
Primer 0.4 µM – LI1 (938 bp) with sequences of: CTC CAT AAA CGT GAT CCT	0.5
Primer 0.4 µM – U1 (938 bp) with sequences of: CAG CMG CCG CGG TAA TWC	0.5
Primer 0.2 µM – LM1 (702 bp) with sequences of: CCT AAG ACG CCA ATC GAA	0.5
Primer 0.2 µM – LM2 (702 bp) with sequences of: AAG CGC TTG CAA CTG CTC	0.5
Sterilized Distilled Water	14.0
DNA Template	2.0
Total	25.0

3.7 Classification of *L. monocytogenes*

Purified *L. monocytogenes* (from section **3.5**) were classified based on its serotype and virulence factors via multiplex polymerase chain reaction (m-PCR).

3.7.1 Serotypes

Five primers pairs, including forward (F) and reverse (R): a) *lmo*0737, sequences of F-5' AGG GCT TCA AGG ACT TAC CC 3' and R-5' ACG ATT TCT GCT TGC CAT TC 3'; b) *lmo*1118, sequences of F-5' AGG GGT CTT AAA TCC TGG AA 3' and R-5' CGG CTT GTT CGG CAT ACT TA 3'; c) OFR2819, sequences of F-5' AGC AAA ATG CCA AAA CTC GT 3' and R-5' CAT CAC TAA AGC CTC CCA TTG 3'; d) ORF2110, sequences of F-5' AGT GGA CAA TTG ATT GGT GAA 3' and R-5' CAT CCA TCC CTT ACT TTG GAC 3' and e) *prs* F-5' GCT GAA GAG ATT GCG AAA GAA G 3' and R-5' CAA AGA AAC CTT GGA TTT GCG G 3' were used for serotyping (**Table 3.3**). These

primer pairs composed of serovar-specific gene to classify *L. monocytogenes* strains into five phylogenetic groups and serovars: a) I.1 (1/2a-3a), which generate one amplification fragment of 691 bp from gene *lmo*0737, b) I.2 (1/2c-3c), which generate two amplification fragments of 691 bp and 906 bp from genes *lmo*0737 and *lmo*1118, respectively, c) II.1 (4b-4d-4e), which generate one amplification fragment of 471 bp from gene ORF2819 d) II.2 (1/2b-3b-7), which generate two amplification fragments of 471 bp and 597 bp from gene ORF2819 and ORF2110, respectively and e) III (4a-4c), which generate one amplification fragment of 370 bp from gene *prs*. All primer pairs were synthesized by Apical Scientific Sdn. Bhd. in Malaysia.

PCR was performed based on the procedures described by Doumith et al. (2004) with slight modifications. Reaction mixture of 25 µL was prepared, containing 5 µL of 1× PCR buffer, 2 µL of 2 mM $MgCl_2$, 0.5 µL of 0.2 mM dNTPs mix, 0.1 µL of 0.5U Taq polymerase, 1.25 µL of 1 µM forward and reverse primers of gene *lmo*0737, ORF2819 and ORF2110, 1.875 µL of 1.5 µM forward and reverse primer of gene *lmo*1118, 0.25 µL of 0.2 µM forward and reverse primer of gene *prs*, 4.65 µL sterile distilled water and 1.0 µL DNA template (from section **3.5**) (**Table 3.3**). All the reagents used were obtained from Promega (Research Instruments, USA).

The PCR cycling condition used: initial denaturation at 94ºC for 3 min, followed by 35 cycles of denaturation at 94ºC for 24 s, annealing at 53ºC for 1.15 min and extension at 72ºC for 1.15 min, followed by a final extension step at 72ºC for 7 min in Thermal Cycler (Matrioux, Malaysia). Then, the PCR products were subjected to 2.0% agarose gel electrophoresis in 0.5× of Tris-Borate-EDTA (TBE) buffer at 100V for 50 min. The gel was then stained with

3× gel red (Biotium, US) and visualized under a gel imager (Bio-rad, USA). A 100 bp Plus DNA ladder (Vivantis Technologies, Malaysia) was used as a DNA marker to estimate the size of amplified PCR products.

Table 3.3: The PCR mixtures with primers' sequences used for serotyping characterization.

Reagent	Amount (µL)
1× PCR Buffer	5.0
2 mM $MgCl_2$	2.0
0.2 mM Deocynucleoside Triphosphate Mix	0.5
0.5U Taq Polymerase	0.1
Primer 1 µM – *lmo*0737 (691 bp) with sequences of:	
F: AGG GCT TCA AGG ACT TAC CC	1.25
R: ACG ATT TCT GCT TGC CAT TC	1.25
Primer 1.5 µM – *lmo*1118 (906 bp) with sequences of:	
F: AGG GGT CTT AAA TCC TGG AA	1.875
R: CGG CTT GTT CGG CAT ACT TA	1.875
Primer 1 µM – OFR2819 (471 bp) with sequences of:	
F: AGC AAA ATG CCA AAA CTC GT	1.25
R: CAT CAC TAA AGC CTC CCA TTG	1.25
Primer1 µM – ORF2110 (597 bp) with sequences of:	
F: AGT GGA CAA TTG ATT GGT GAA	1.25
R: CAT CCA TCC CTT ACT TTG GAC	1.25
Primer 0.2 µM – *prs* (370 bp) with sequences of:	
F: GCT GAA GAG ATT GCG AAA GAA G	0.25
R: CAA AGA AAC CTT GGA TTT GCG G	0.25
Sterilized Distilled Water	4.65
DNA Template	1.0
Total	25.0

F – forward primer; R – reverse primer

3.7.2 Virulence Genes

Two sets of virulence gene markers were implemented for the characterization of purified *L. monocytogenes* (from section **3.5**). Internalin gene A (*inl*A), C (*inl*C) and J (*inl*J) was identified in the first set of analysis, whereas *plc*A, *act*A, *hly*A and *iap* genes were implicated in the second set of analysis.

In the first set of analysis, three primer pairs were used to determine the virulence genes of *L. monocytogenes*: a) *inl*A, sequences of F-5' ACG AGT AAC GGG ACA AAT GC 3' and R-5' CCC GAC AGT GGT GCT AGA TT 3', product size of 800 bp; b) *inl*C, sequences of F-5' AAT TCC CAC AGG ACA CAA CC 3' and R-5' CGG GAA TGC AAT TTT TCA CTA 3', product size of 517 bp and c) *inl*J, sequences of F-5' TGT AAC CCC GCT TAC ACA GTT 3' and R-5' AGC GGC TTG GCA GTC TAA TA 3', product size of 238 bp (**Table 3.4**). All three primer pairs targeted genes encoded for surface-associated internalin found in *L. monocytogenes*, which is correlated to its pathogenicity towards human listeriosis. All primer pairs were synthesized by Apical Scientific Sdn. Bhd. in Malaysia.

m-PCR was prepared based on the procedures described by Liu et al. (2007) with slight modifications. m-PCR reaction was carried out in a total volume of 25 µL which comprising of 5 µL of 1× PCR buffer, 2 µL of 2mM $MgCl_2$, 0.5 µL of 0.2mM dNTPs mix, 0.25 µL of 1.25U of Taq polymerase, 1 µL of 0.4 µM forward and reverse primer of *inl*A, 0.75 µL of 0.3 µM forward and reverse primer of *inl*C, 0.5 µL of 0.2 µM forward and reverse primer of *inl*J, 10.75 µL sterile distilled water and 2 µL DNA template (from section **3.5**) (**Table 3.4**). All the reagents were obtained from Promega (Research Instruments, USA).

The PCR conditions used was as such: initial denaturation at 94ºC for 2 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s and extension at 72ºC for 1 min, followed by a final extension step at 72ºC for 10 min using Thermal Cycler (Matrioux, Malaysia). Then, the PCR products were separated using gel electrophoresis in 1.5% agarose gel in 0.5× of Tris-Borate-EDTA (TBE) buffer at 100V for 35 min. After electrophoresis,

the gel was stained with 3× gel red (Biotium, US) and visualized under a gel imager (Bio-rad, USA). A 100 bp Plus DNA ladder (Vivantis Technologies, Malaysia) was used as a DNA marker to estimate the size of amplified PCR products.

On the other hand, in the second set of analysis, four primer pairs were used to determine the virulence genes of *L. monocytogenes*: a) *plc*A, sequences of F-5'CTG CTT GAG CGT TCA TGT CTC ATC CCC C 3' and R-5' ATG GGT TTC ACT CTC CTT CTA C 3', product size of 1484 bp; b) *act*A, sequences of F-5'CGC CGC GGA AAT TAA AAA AAG A 3' and R-5'ACG AAG GAA CCG GGC TGC TAG 3', product size of 839 bp; c) *hly*A, sequences of F-5'GCA GTT GCA AGC GCT TGG AGT GAA 3' and R-5' GCA ACG TAT CCT CCA GAG TGA TCG 3', product size of 456 bp and d) *iap*, sequences of F-5' ACA AGC TGC ACC TGT TGC AG 3' and R-5' CAG CGT GTG TAG TAG CA 3', product size of 131 bp (**Table 3.5**). All of four primer pairs are targeted virulence-associated genes that causing pathogenesis of *L. monocytogenes*. All primer pairs were synthesized by Apical Scientific Sdn. Bhd. in Malaysia.

m-PCR was performed according to the procedures described by Rawool et al. (2007) with slight modifications. 25 µL of mixture of reagents comprising 5 µL of 1× PCR buffer, 6 µL of 6mM $MgCl_2$, 2.5 µL of 1mM dNTPs mix, 0.4 µL of 4U of Taq polymerase, 0.25 µL of 0.1 µM forward and reverse of each primer pairs, 6.6 µL sterile distilled water and 2.5 µL DNA template (from section **3.5**) (**Table 3.5**). All the reagents used were purchased from Promega (Research Instruments, USA).

Thermal cycling conditions used was as such: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at

60ºC for 30 s and extension at 72ºC for 1.5 min, followed by a final extension step at 72ºC for 10 min in Thermal Cycler (Matrioux, Malaysia). Then, the PCR products were separated using gel electrophoresis in 1.5% agarose gel in 0.5× of Tris-Borate-EDTA (TBE) buffer at 100V for 40 min. After electrophoresis, the gel was stained with 3× gel red (Biotium, US) and visualized under a gel imager (Bio-rad, USA). A 100 bp Plus DNA ladder (Vivantis Technologies, Malaysia) was used as a DNA marker to estimate the size of amplified PCR products.

Table 3.4: The PCR mixtures with primers' sequences used in virulence gene markers of *inl*A, *inl*C and *inl*J identification of *L. monocytogenes*.

Reagent	Amount (µL)
1× PCR Buffer	5.0
2 mM $MgCl_2$	2.0
0.2 mM Deocynucleoside Triphosphate Mix	0.5
1.25U Taq Polymerase	0.25
Primer 0.4 µM – *inl*A (800 bp) with sequences of:	
F: ACG AGT AAC GGG ACA AAT GC	1.0
R: CCC GAC AGT GGT GCT AGA TT	1.0
Primer 0.3 µM – *inl*C (517 bp) with sequences of:	
F: AAT TCC CAC AGG ACA CAA CC	0.75
R: CGG GAA TGC AAT TTT TCA CTA	0.75
Primer 0.2 µM – *inl*J (238 bp) with sequences of:	
F: TGT AAC CCC GCT TAC ACA GTT	0.5
R: AGC GGC TTG GCA GTC TAA TA	0.5
Sterilized Distilled Water	10.75
DNA Template	2.0
Total	25.0

F – forward primer; R – reverse primer

Table 3.5: The PCR mixtures with primers' sequences used in virulence gene markers of *plc*A, *act*A, *hly*A and *iap* identification of *L. monocytogenes*.

Reagent	Amount (µL)
1× PCR Buffer	5.0
6 mM $MgCl_2$	6.0
1 mM Deocynucleoside Triphosphate Mix	2.5
4U Taq Polymerase	0.4
Primer 0.1 µM – *plc*A (1484 bp) with sequences of:	
F: CTG CTT GAG CGT TCA TGT CTC ATC CCC C	0.25
R: ATG GGT TTC ACT CTC CTT CTA C	0.25
Primer 0.1 µM – *act*A (839 bp) with sequences of:	
F: CGC CGC GGA AAT TAA AAA AAG A	0.25
R: ACG AAG GAA CCG GGC TGC TAG	0.25
Primer 0.1 µM – *hly*A (456 bp) with sequences of:	
F: GCA GTT GCA AGC GCT TGG AGT GAA	0.25
R: GCA ACG TAT CCT CCA GAG TGA TCG	0.25
Primer 0.1 µM – *iap* (131 bp) with sequences of:	
F: ACA AGC TGC ACC TGT TGC AG	0.25
R: CAG CGT GTG TAG TAG CA	0.25
Sterilized Distilled Water	6.6
DNA Template	2.5
Total	25.0

F – forward primer; R – reverse primer

3.8 Determination of Antibiotic Resistance Profiles

The antibiotic resistance profile of *L. monocytogenes* (from section **3.7**) was determined using Kirby-Bauer disk diffusion method (CLSI, 2020). A total of 13 antibiotics were used consisting of 9 different families: beta-lactams (amoxicillin/clavulanic acid (AMC, 30 µg), oxacillin (OX, 1 µg), penicillin (P, 10 µg), ampicillin (AMP, 10 µg)); aminoglycosides (gentamicin (CN, 10 µg), streptomycin (S, 25 µg)); macrolides (erythromycin (E, 15 µg)); fluoroquinolones (ciprofloxacin (CIP, 5 µg)); sulfonamides (sulfamethoxazole/trimethoprim (SXT, 25 µg)); rifamycin (rifampicin (RD, 5 µg)); tetracycline (tetracycline (TE, 30 µg)); carbapenem (meropenem (MEM, 10 µg)) and lincosamide (clindamycin (DA, 2 µg)) (Oxoid, UK).

Briefly, the bacteria were streak-plated on TSA plate and incubated for 24 h at 37°C. After incubation, three to five colonies were picked and suspended in 5 ml of sterile saline, 0.85% of NaCl. The suspension was adjusted to turbidity of 0.5 McFarland using a McFarland densitometer (Biosan, US). Adjusted suspension was then spread evenly onto a 4 mm depth of Muller Hinton agar using a sterile cotton bud. Suspension was allowed to dry for 3 to 5 mins before the antibiotic disc was placed onto the agar with a disc dispenser and incubated for 24 h at 37°C. Reference strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as negative controls throughout the study.

Antibiotic resistance profile of *L. monocytogenes* isolates was evaluated by measuring the inhibition zone (diameter at the nearest millimetre). Interpretation of inhibition zone for susceptibility, intermediate and resistance was based on the breakpoint's guidelines from Clinical Laboratory Standards Institute (CLSI, 2020) and European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) of *L. monocytogenes*, except for those antibiotics which were not listed, the breakpoint was interpreted based on the zone inhibition generated by *Staphylococcus* spp.

Multiple antibiotic resistance (MAR) phenotypes pattern was identified using the formula of MAR index = a/b as described by Krumperman (1983), whereby "a" indicates the number of antibiotics found to be resistant by particular isolate and "b" indicates the sum number of the tested antibiotics.

3.9 Preparation of Test Coupons for Biofilm Formation

3.9.1 Stainless-Steel

Grade 304 stainless-steel with finishing of no. 4 (#4) and 2B were purchased from SMF Steelmakers Sdn. Bhd., Malaysia and used as test coupons. The coupons were cut into size of 1.5 × 1.5 × 0.1 cm prior to cleaning and sanitizing. Cleaning and sanitization procedures were carried out based on Oliveira et al. (2010) and Pérez-Ibarreche et al. (2016) with slight modification. Briefly, a test coupon was soaked with 99.5% of acetone for 1 h before rinsing with distilled water. Then, it was soaked in 1 M of sodium hydroxide (NaOH) and 95% of ethanol, respectively for 1 h prior to rinsing with distilled water. It was transferred to 50 mL falcon tube for drying at 70°C and autoclaving at 121°C for 15 min. The test coupon was dried overnight prior usage.

3.9.2 Teflon

Teflon sheet was purchased from HardwareMISE Sdn. Bhd., Malaysia and was cut into pieces in size of 1.5 × 1.5 × 0.1 cm. It was cleaned and sanitized as described in section **3.9.1**.

3.9.3 Plastic

Plastic sheets of high-density polyethylene (HDPE) and polypropylene (PP) were purchased from Euroshore Sdn. Bhd., Malaysia. HDPE test coupon was cut into size of 1.5 × 1.5 × 0.2 cm, whereas PP test coupon was cut into size of 1.5 × 1.5 × 0.3 cm. Both plastic materials were cleaned and sanitized based on procedures described in section **3.9.1**, except autoclaving.

3.10 Biofilm Study

Isolate from the most common *L. monocytogenes* serotype group from **section 3.6**, possessed most virulence genes encoding all seven virulence genes of *inl*A, *inl*C, *inl*J, *plc*A, *act*A, *hly*A and *iap* gene in **section 3.7** and showed high resistance towards antibiotics (with MAR index that higher than 0.2) in **section 3.8** which able to cause human listeriosis was chosen and used in the biofilm study. ATCC 19112 of *L. monocytogenes* was used as positive control in the study.

3.10.1 Preparation of OD-adjusted *L. monocytogenes*

Stock culture of *L. monocytogenes* was streak-plated on TSA plate and incubated for 24 h at 37ºC. Colonies from TSA plate was picked and suspended into 15 ml falcon tube filled with 10 ml of sterile Tryptic Soy Broth (TSB). The suspension was mixed well using vortex and further incubated for 18 to 20 h, 37ºC at 200 rpm. Overnight culture was then adjusted to OD_{600} 0.393±0.02 with sterile TSB (Giaouris et al., 2009). Serial dilution was performed up to 10^{-6}. Each dilution was spread-plated on TSA agar in replicates and incubated for 24 h at 37ºC. The cell count was confirmed to be in the range of 8 $\log_{10}$ CFU/ml prior to further analyzes.

3.10.2 Growth curve of *L. monocytogenes*

3.10.2.1 Tryptic Soy Broth (TSB)

Nine tubes of 5 ml OD-adjusted suspension of overnight culture were prepared (section **3.10.1)** and incubated at 37ºC without agitation. After incubation time of 1 h, one tube was removed and mixed. The density of *L. monocytogenes* in

TSB was determined by measuring the optical density of suspension at wavelength of 600 nm with microtiter plate. Three readings were taken at each time point. The steps were repeated at regular 1 h interval for up to 9 h of incubation time. Average of readings was calculated at each time point and a graph of OD versus time was generated.

3.10.2.2 Test Surfaces

Twenty-one of cleaned and sanitized grade 304 stainless-steel coupons were prepared as described in section **3.9.1** and placed into a sterile 12 wells culture plates. 2 ml of OD adjusted suspension of overnight *L. monocytogenes* culture [OD_{600} 0.393±0.02 (8 $\log_{10}$ CFU/mL)] (section **3.10.1)** were transferred into each well of the culture plate. Culture plates was then incubated at 37ºC for 3 h, 6 h, 24 h, 48 h, 72 h, 96 h and 120 h without agitation. The arrangement for each test surfaces in the 12 wells culture plate, are shown in **Figure 3.3.** Three coupons were collected for each time point. Inoculum was then removed from each well. Test surface was washed three times in 0.1× phosphate buffer solution (PBS) before the coupon was transferred to a 50 mL falcon tube containing 10 mL of sterile 0.85% saline solution. The falcon tube was vortexed vigorously for 2 min with a bench-top vortex at a maximum speed. Suspension was serial diluted between 10^{-1} to 10^{-6}. Each dilution was spread-plated on TSA agar in duplicates. The step was repeated at each time point. After incubation, CFU was calculated and expressed in $\log_{10}$ CFU/cm^2. A graph of log CFU/cm^2 versus time was generated.

Figure 3.3: Test coupons arrangement in 12 wells culture plate and its labelling based on the incubation time (3 h, 6 h, 24 h, 48 h, 72 h, 96 h and 120 h) and replicated in thrice.

3.10.3 Preparation of Inoculums for Biofilm Formation

Frozen stock culture of *L. monocytogenes* (ATCC 19112 and isolated positive environmental isolate) was thawed and streak-plated on TSA agar. The culture was incubated for 24 h at 37ºC. Colonies was scraped with a sterile loop from the plate and inoculated into a 15 mL falcon tube filled with 10 mL of TSB. The mixture was homogenized evenly with a vortex and incubated in a shaking incubator (Infors, Switzerland) for 18-20 h, 37ºC at speed of 200 rpm. Overnight culture was vortexed and then transferred into a new 50 mL falcon tube for dilution with fresh TSB. Bacteria suspension was vortexed evenly before measuring the absorbance value. The absorbance of the bacteria suspension was adjusted to OD_{600} 0.393±0.02 (8 $\log_{10}$ CFU/mL) using microtiter plate reader (FLUOstar Omega, BMG Labtech, Germany).

3.10.4 Biofilm Formation on Test Surface

Adjusted bacteria suspension (section **3.10.3**) was then used as an inoculum for biofilm formation on test surfaces. Each test surface was performed in triplicates and the procedures was repeated twice. Thus, the number of replicates for each test surface was n=6. The arrangement for each test surfaces in the 12 wells culture plate, are shown in **Figure 3.4** or **Figure 3.5**. The configuration of test surfaces without treatment is shown in **Figure 3.4**. On the other hand, **Figure 3.5** illustrated the arrangement of test surfaces with treatment, in which the adhered *L. monocytogenes* biofilm on each test surface was subsequently treated with different types of disinfectant and sanitizing solutions in order to remove the biofilm.

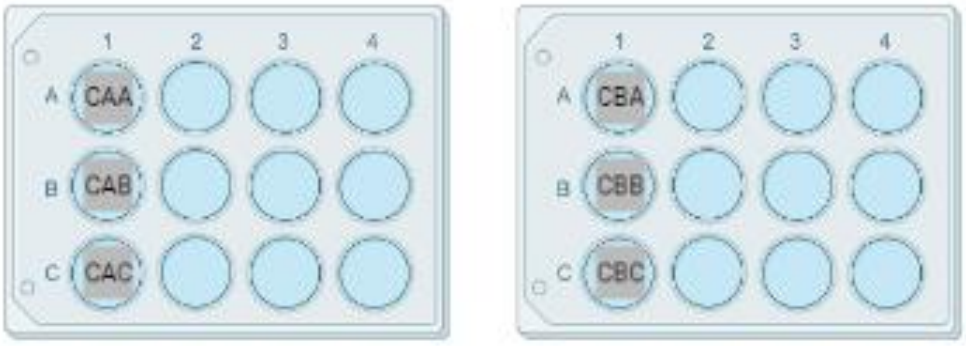


Figure 3.4: Test coupons arrangement in 12 wells culture plate and its labelling. (Set 1: CAA, CAB and CAC; Set 2: CBA, CBB and CBC). This is the arrangement used for *L. monocytogenes* with no treatment. CAA, CAB and CAC were the first set of triplicate test surface without treatment; CBA, CBB and CBC were the second first set of triplicate test surface without treatment.

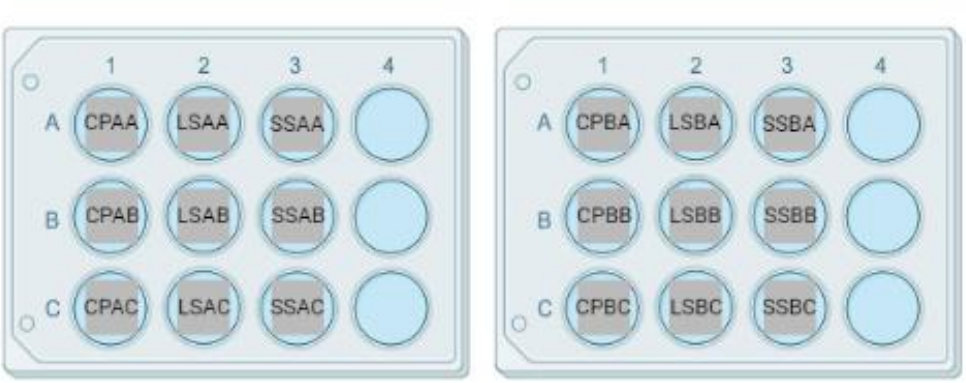


Figure 3.5: Test coupons arrangement in 12 wells culture plate and its labelling. (Set 1: CPAA, CPAB, CPAC, LSAA, LSAB, LSAC, SSAA, SSAB and SSAC; Set 2: CPBA, CPBB, CPBC, LSBA, LSBB, LSBC, SSBA, SSBB and SSBC). This the arrangement used for *L. monocytogenes* with treatment. CPAA, CPAB and CPAC were the first set of triplicate test surface with COS PAA (CP) treatment; LSAA, LSAB and LSAC were the first set of triplicate test surface with Liquid Sanitizer (LS) treatment and SSAA, SSAB and SSAC were the first set of triplicate test surface with SS Sanitizer (SS) treatment. CPBA, CPBB and CPBC were the second set of triplicate test surface with CP treatment; LSAA, LSAB and LSAC were the second set of triplicate test surface with LS treatment and SSAA, SSAB and SSAC were the second set of triplicate test surface with SS treatment.

Cleaned and autoclaved test coupons were placed individually to fill in 12 wells culture plates. A 2 mL adjusted bacteria suspension (from section **3.10.3**) was then transferred into each well. The culture plate was then sealed with parafilm and incubated for 6 h at 4ºC, 25ºC, 30ºC and 37ºC without agitation.

3.10.5 Enumeration of *L. monocytogenes* on Test Surface

The number of *L. monocytogenes* adhered to the test surface was determined after 6 h of incubation (as section **3.10.4**). The inoculum was then removed from each well. Test surface was immersed three times in 0.1× PBS to remove unattached planktonic cells. It was then transferred to a 50 mL falcon tube containing 10 mL of sterile 0.85% of saline solution. The falcon tube was vortexed vigorously for 2 min with a bench-top vortex at a maximum speed to

remove adhered cell on the test surface. The suspension was then diluted to a factor of 10^{-6} with sterile 0.1% of peptone water in 900 µL of micro-centrifuge tube. A 10 µL of diluted suspension from each dilution factor was dropped onto TSA agar (**Figure 3.6**) and allowed to air dried before incubated for 24 h at 37ºC (Chen et al. 2003).

Figure 3.6: Enumeration of *L. monocytogenes* using drop plate method for each test surface. Dilution factor of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} was prepared from left to right.

The incubated plates were viewed under stereo microscope (Motic, HK). Droplet with at least 3 to 30 colonies per 10 µL were considered as a countable dilution range (30 to 300 colonies per 100 µL). Colony forming unit (CFU) for each test surface was calculated based on the formula described by Herigstad et al. (2001) and Christen and Parker (2020) (**Figure 3.7)**. Results were expressed as the average of both triplicates (n=6) ± standard deviation in $\log_{10}$ CFU/cm^2.

$$CFU = \frac{average\ of\ countable\ cell\ number}{volume\ of\ each\ droplet} \times countable\ dilution\ factor \times \frac{volume\ of\ new\ tube}{surface\ area}$$

Figure 3.7: Calculation of CFU after serial dilution. Formula was derived from Herigstad et al. (2001), Chen et al. (2003) and Christen and Parker (2020) with slight modifications.

The procedures of section **3.10.3** to section **3.10.5** were applied in all test surface groups of stainless-steel (#4 and 2B), Teflon and plastic (HDPE and PP).

3.10.6 Removal of *L. monocytogenes* Biofilm on Test Surfaces

Three types of disinfectant and sanitizing solutions were used to remove the biofilm of *L. monocytogenes*: a) acidic based compound (COS PAA), b) chlorine-based compound (Liquid Sanitizer) and c) a mixture of quaternary ammonium compounds (QAC) (SS Sanitizer). These solutions were purchased from Cosmic Discovery Sdn. Bhd., Malaysia. The stainless-steel (finishing of #4 and 2B) were immersed with solutions diluted with distilled water at 200 ppm; Teflon was immersed at 100 ppm and the plastic test materials (HDPE and PP) surfaces were immersed with the solutions diluted with distilled water at 50 ppm, respectively.

3.10.7 Viable Cell of *L. monocytogenes* on Test Surfaces After Treatment

Viable cell number of *L. monocytogenes* adhered on test surface was determined after treated with the solutions described in section **3.10.5**. The inoculum was removed from each well of culture plate (**Figure 3.4**). Test surface was immersed three times in 0.1× PBS to remove unattached planktonic cells.

A 1 mL of each diluted disinfectant and sanitizing solution corresponding to different test surfaces (section **3.10.6**) was added to each well for 1 min before discarded. Then 2 mL of sterile neutralizing solution comprises of TSB, 0.3% lecithin (Himedia, India) and 2% Tween 80 (Merck, Germany), was applied to the test surfaces for 10 min to inactivate the disinfectant activity of the solution. This was then followed by washing with 1 mL of 0.1× PBS (Kawakami et al. 2010).

Adhered cell on a treated test surface was then transferred to a 50 mL falcon tube containing 10 mL of sterile 0.85% of saline solution and was vortexed for 2 min. The suspension was diluted to a factor of 10^{-6} by transferring 100 µL of the first suspension to 900 µL of 0.1% peptone water in 1.5 mL micro-centrifuge tubes. A 10 µL of the diluted suspension from each dilution factors of 10^{-1} to 10^{-6} was dropped plated onto TSA agar and allowed to air-dry before incubated for 24 h at 30^{o}C.

Cell count of each droplet was enumerated through stereo microscopy viewing. CFU was calculated based on the formula stated in section **3.10.5**. Each test surface material was conducted in triplicates and repeated twice. Results were analyzed based on the average of both triplicates (n=6) ± standard deviation in log CFU/cm^2.

The procedures of section **3.10.3** to section **3.10.5** were applied in all test surface groups of stainless-steel (#4 and 2B), Teflon and plastic (HDPE and PP).

3.10.8 Effectiveness of Disinfectant and Sanitizing Solution to Biofilm of *L. monocytogenes*

The effectiveness of the disinfectant and sanitizing solutions towards the biofilm of *L. monocytogenes* are calculated based on the formula shown in **Figure 3.8**.

$$Reduction = Log10\ (Initial\ viable\ cell\ capcity - Viable\ cell\ capacity\ remaining)$$

Figure 3.8: **The reduction of cell count can estimate the effectiveness of the disinfectant and sanitizing solutions.** Initial viable cell capacity could be obtained from section **3.10.5** and remaining viable cell capacity could be obtained from section **3.10.7.**

3.11 Scanning Electron Microscopy (SEM)

Test surfaces grew under various temperature of 4ºC, 25ºC, 30ºC and 37ºC with and without treatment were chosen to perform visualization in microscopy level using field emission scanning electron microscope (JEOL JSM-6701-F, JEOL, Japan).

Test coupons of stainless steel with finishing no. 4 (#4) were prepared according to the steps carried out in section **3.10.4** and **3.10.7**. They were air-dried prior to the fixation of 2.5% glutaraldehyde in 0.1× of PBS overnight at 4ºC before rinsing with 0.1× PBS for 10 min each. This step was repeated three times. Then, dehydration steps were performed using an ascending series of ethanol (50, 75, and 95%) on the test coupon surfaces for 10 min prior washing with absolute ethanol (100%) for three times. The coupons were air-dried and sputter coated with a thin layer of platinum using an auto fine coater (JFC-1600, JEOL, Japan) before sending for scanning electron microscope analysis (JEOL JSM-6701-F, JEOL, Japan) (Lee et al., 2019).

3.12 Statistical Analysis of Data

Statistical analysis of data collected was performed using SPSS version 26.0. Results are considered statistically significant when ρ-value is less than 0.05 ($\rho<0.05$). Chi-square test and Fisher exact test were implemented to determine the significance difference between the prevalence of *Listeria* spp. and *L. monocytogenes* in both sources (food and food contact surface) of isolates. Two-way analysis of variance (ANOVA) with Levene's test as homogeneity determinator was carried out to investigate the interactions between the independent variables: a) surface type, temperature and disinfectant and sanitizing solution used; b) surface type and disinfectant and sanitizing solution used and the dependent variable a) growth of cell in CFU count; b) reduction of cell in CFU count, respectively. Multiple comparison of Tukey's Honestly Significant Difference (HSD) post hoc test was used to evaluate the difference of each independent variables and dependent variable.

CHAPTER 4

RESULTS

4.1 Prevalence of *Listeria* in food and food contact surfaces

The presence of *L. monocytogenes* in food and food contact surfaces was detected on PALCAM agar which exhibited colonies of grey-green colour with black centre (**Figure 4.1**), whereas on TSA agar, it showed the presence of milky white colonies (**Figure 4.2**). These positive colonies were verified using d-PCR (**Figure 4.3**).

The prevalence of *Listeria* spp. and *L. monocytogenes* in food and on food contact surfaces are tabulated in **Table 4.1**. Out of the 322 total samples, *Listeria* was discovered in 69 (21.42%) of the collected samples. More than half of these samples (n=41/69, 59.42%) were tested positive for *L. monocytogenes*. Food sources (n=26/170, 15.29%) were likely to be contaminated by *L. monocytogenes* compared to food contact surfaces (n=15/152, 9.87%). Among the four food sources, processed food had the highest prevalence at 33.33% (n=2/6), followed by minimally processed food which made up 31.25% (n=10/33), raw food at 26.32% (n=10/38) and ready-to-eat (RTE) food at 4.26% (n=4/93), of the 26 *L. monocytogenes* positive food samples. On the other hand, the prevalence of *L. monocytogenes* was observed to be higher on surfaces directly in touch with food (n=11/93, 11.83%) compared to indirect food contact surfaces (n=4/59, 6.78%). There was notable significant difference in the prevalence of *Listeria* spp. and *L. monocytogenes* between the food and food

contact surface isolates (Pearson Chi-Square=28.69, ρ=0.000). Similarly, the prevalence of *Listeria* spp. and *L. monocytogenes* among the four types of food: raw food, minimally processed food, processed food and ready-to-eat food, was found significantly different in Pearson Chi-Square=13.53, ρ=0.004. However, the prevalence of *Listeria* spp. and *L. monocytogenes* between the direct and indirect food contact surface was not significantly difference, ρ=0.466 in Fisher's Exact Test.

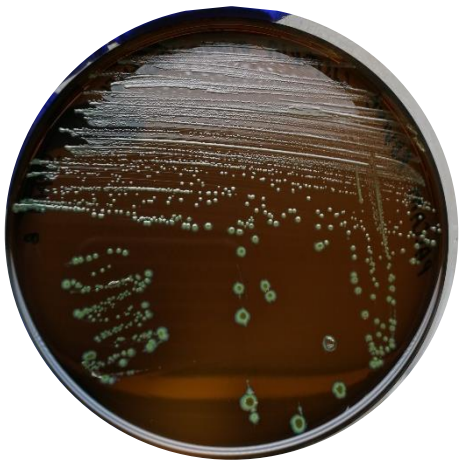


Figure 4.1: The grey-green colonies with black centre on PALCAM agar exhibited the presence of *L. monocytogenes*.

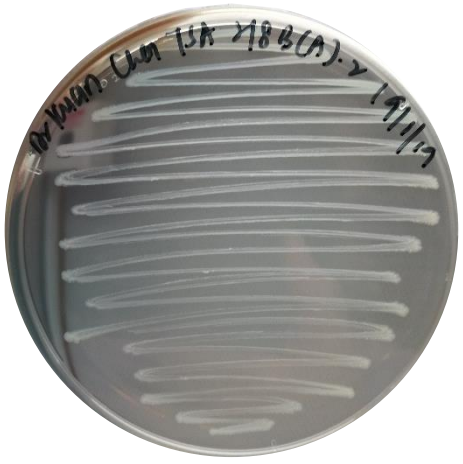


Figure 4.2: The growth of milky white colonies on TSA agar showing the presence of *L. monocytogenes*.

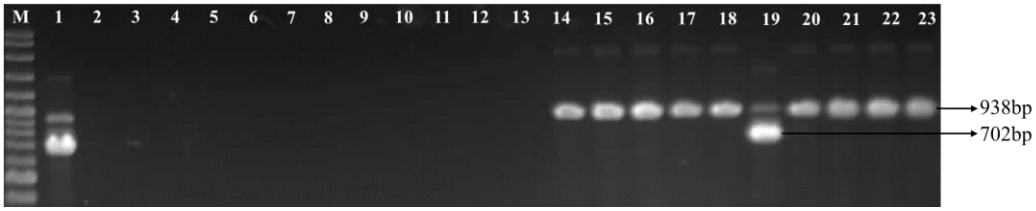


Figure 4.3: The 1.5% gel electrophoresis showed the d-PCR amplification of genes 16S rRNA (938bp) and *hly*A (702 bp) for the detection of *Listeria* spp. and *Listeria monocytogenes*, respectively. Lane M: 100 bp Plus DNA ladder partially similar as complete 1 kb ladder (Vivantis Technologies, Malaysia); Lane 1: *L. monocytogenes* ATCC 19112 strains (positive control); Lane 2: Distilled water as template (non-template control); Lane 3 to 13: *Listeria* spp. negative samples; Lane 14 to 18: *Listeria* spp. positive samples; Lane 19: *L. monocytogenes* positive sample; Lane 20 to 23: *Listeria* spp. positive samples.

Table 4.1: Prevalence of *Listeria* spp. and *L. monocytogenes* in both sources of isolates.

Source of isolates	No. of samples	No. (%) of *Listeria* spp.	No. (%) of *L. monocytogenes*
Food	**170**	**49 (28.82) ^{A}**	**26 (15.29) ^{A}**
Raw Food	38	14 (36.84) ^{a}	10 (26.32) ^{a}
Minimally Processed Food	33	16 (48.48) ^{b}	10 (31.25) ^{b}
Processed Food	6	2 (33.33) ^{a}	2 (33.33) ^{a}
Ready-to-eat Food	93	17 (18.28) ^{b}	4 (4.26) ^{b}
Food Contact Surface	**152**	**20 (13.16) ^{B}**	**15 (9.87) ^{B}**
Direct Food Contact	93	14 (15.05) ^{a}	11 (11.83) ^{a}
Indirect Food Contact	59	6 (10.17) ^{a}	4 (6.78) ^{a}
Total	**322**	**69 (21.43)**	**41 (12.73)**

$*^{A-B}$ indicate within the same column with the same superscripts are not significantly different ($\rho>0.05$) in overall of food and food contact surface sources while data with different superscripts are significantly different ($\rho<0.05$) in overall of food and food contact surface sources.

$*^{a-b}$ indicate within the same column with the same superscripts are not significantly different ($\rho>0.05$) in respective group sources while data with different superscripts are significantly different ($\rho<0.05$) in respective group sources.

4.2 Classification of *L. monocytogenes*

4.2.1 Serotype

A total of 351 *L. monocytogenes* isolates were collected from positive samples in food and on food contact surface and its serotypes was tabulated in **Table 4.2** after the pre-determination by m-PCR (**Figure 4.4**). However, 23 out of 351

isolates were unable to detect. Only 313 out of 328 was able to detect and classified into 5 serogroups. The highest prevalent of serotypes detected in the positive samples was group III (4a-4c) shows the highest prevalence 44.09% (138/313), followed by serogroup of II.2 (1/2b-3b-7) at 30.03% (94/313), serogroup I.1 (1/2a-3a) at 14.70% (46/313), serogroup II.1 (4b-4d-4e) at 7.99% (25/313) and the least detected was serogroup I.2 (1/2c-3c) at 3.19% (10/313). The serotypes identified was mainly derived from food sources (277) instead of the food contact surfaces (36). It was observed that the prevalence of isolates in each serogroup from food sources was higher than that of isolates in each serogroup from food contact surfaces except serogroup II.2 (1/2b-3b-7). **Table 4.3** summarizes the overall prevalence of serotype of *L. monocytogenes* among four groups of food and between two groups of food contact surface.

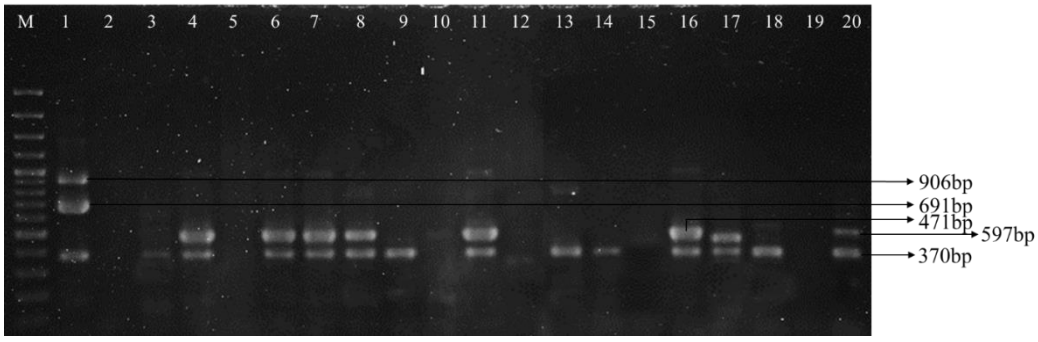


Figure 4.4: The image represented 1.5% gel electrophoresis showed the m-PCR amplification of genes *lmo*1118 (906 bp), *lmo*0737 (691 bp), ORF2110 (597 bp), ORF2819 (471 bp) and *prs* (370 bp), respectively. Lane M: 100 bp Plus DNA ladder partially similar as complete 1 kb ladder (Vivantis Technologies, Malaysia); Lane 1: *L. monocytogenes* ATCC 19112 strains (positive control) and classified as serogroup of I.2; Lane 2: Distilled water as template (non-template control); Lane 5, 10, 15 and 19: non-amplified *L. monocytogenes* isolate samples; Lane 4, 6, 7, 8, 11, 16 and 17: *L. monocytogenes* isolate samples classified as serogroup of II.2; Lane 18 and 20: *L. monocytogenes* isolate samples classified as serogroup of II.1; Lane 3, 9, 12, 13 and 14: *L. monocytogenes* isolate samples classified as serogroup of III.

Table 4.2: Serotype of *L. monocytogenes* from the food and food contact surfaces.

Source of isolates	No. (%) of *L. monocytogenes* serotype-positive isolates					Total isolates
	I.1 (1/2a-3a)	I.2 (1/2c-3c)	II.1 (4b-4d-4e)	II.2 (1/2b-3b-7)	III (4a-4c)	
Food						
RF	5 (8.77)	0	3 (5.26)	20 (35.09)	29 (50.88)	57
MPF	16 (17.58)	2 (2.20)	4 (4.40)	29 (31.87)	40 (43.96)	91
PF	16 (24.62)	1 (1.54)	5 (7.69)	15 (23.08)	28 (43.08)	65
RTE	4 (6.25)	7 (10.94)	13 (20.31)	13 (20.31)	27 (42.19)	64
Food Contact Surface						
DFCS	2 (25.00)	0	0	2 (25.00)	4 (50.00)	8
IFCS	3 (10.71)	0	0	15 (53.57)	10 (35.71)	28
Total	46 (14.70)	10 (3.19)	25 (7.99)	94 (30.03)	138 (44.09)	313

RF – Raw Food; MPF – Minimally Processed Food; PF – Processed Food; RTE – Ready-to-Eat Food; DFCS – Direct Food Contact Surface; IFCS – Indirect Food Contact Surface

4.2.2 Virulence Genes

Virulence gene was utilized as markers to classify positive isolates of *L. monocytogenes* to understand its pathogenicity. Classification was examined through m-PCR amplification of sequences from two sets of virulence gene groups (**Figure 4.5**; **Figure 4.6**). Internalin gene of *inl*J was discovered to be present in most of the 313 *L. monocytogenes* positive isolates (41.8%). Serogroup I.1 (1/2c-3c) and II.2 (1/2b-3b-7) isolates exhibited to carry all the virulence gene markers at higher prevalent counts when compared to isolates in other serogroups (**Table 4.3**). Out of 313 isolates, 117 isolates carry the virulence gene marker of *hly*A and 50 of them belongs to serogroup II.2 (1/2b-3b-7). The least detected virulence gene marker was *plc*A gene, at the prevalence of 16.93%.

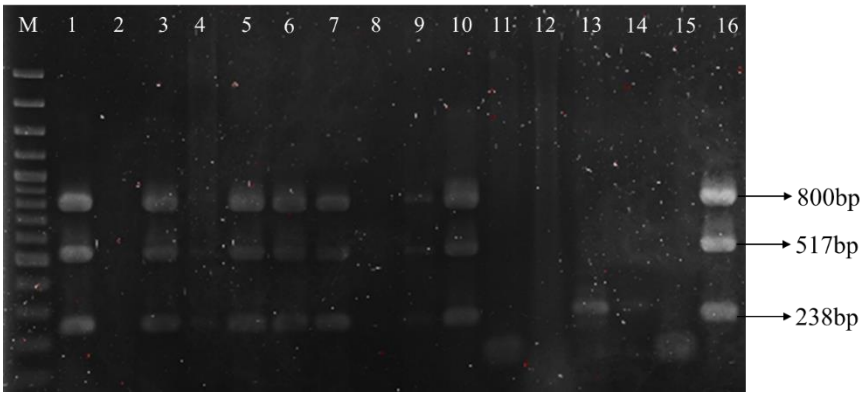


Figure 4.5: The image represented 1.5% gel electrophoresis showed the m-PCR amplification of genes *inl*A (800 bp), *inl*C (517 bp) and *inl*J (238 bp), respectively. Lane M: 100 bp Plus DNA ladder partially similar as complete 1 kb ladder (Vivantis Technologies, Malaysia); Lane 1: *L. monocytogenes* ATCC 19112 strains (positive control) and consisted virulent genes of *inl*A, *inl*C and *inl*J; Lane 2: Distilled water as template (non-template control); Lane 4, 8, 11, 12, 14 and 15: non-amplified *L. monocytogenes* isolate samples without virulent genes of *inl*A, *inl*C and *inl*J; Lane 3, 5, 6, 7, 9, 10 and 16: *L. monocytogenes* isolate samples that consisted of virulent gene of *inl*A, *inl*C and *inl*J; Lane 13: *L. monocytogenes* isolate samples that consisted only one virulent gene of *inl*J.

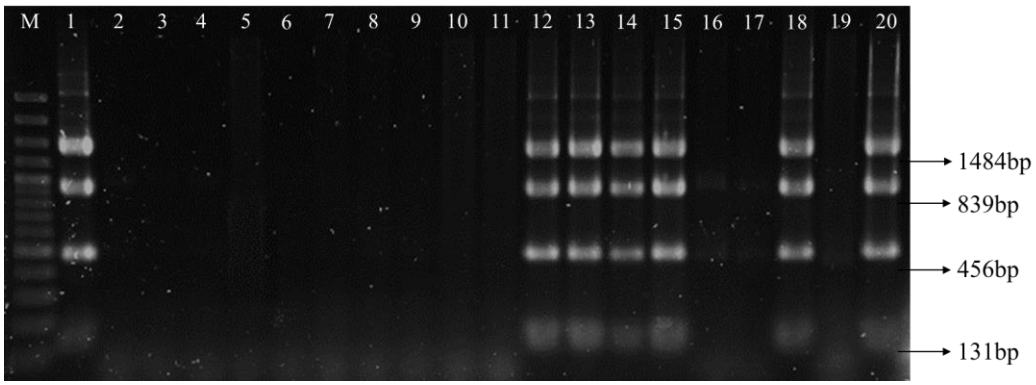


Figure 4.6: The image represented 1.5% gel electrophoresis showed the PCR amplification of genes *plc*A (1484 bp), *act*A (839 bp), *hly*A (456 bp) and *iap* (131 bp), respectively. Lane M: 100 bp Plus DNA ladder partially similar as complete 1 kb ladder (Vivantis Technologies, Malaysia); Lane 1: *L. monocytogenes* ATCC 19112 strains (positive control) and consisted virulent genes of *plc*A, *act*A, *hly*A and *iap*; Lane 2: Distilled water as template (non-template control); Lane 3 to 11, 16, 17 and 18: *L. monocytogenes* isolate samples without virulent genes of *plc*A, *act*A, *hly*A and *iap*; Lane 12 to 15, 18 and 20: *L. monocytogenes* isolate samples that consisted virulent gene of *plc*A, *act*A, *hly*A and *iap*.

Table 4.3: Virulence gene profile of 313 *L. monocytogenes* isolates found in samples that tested positive from both sources.

Virulence Gene	No. (%) of positive isolates	No. (%) of *L. monocytogenes* serotype-positive isolates				
		I.1 (1/2a-3a)	I.2 (1/2c-3c)	II.1 (4b-4d-4e)	II.2 (1/2b-3b-7)	III (4a-4c)
*inl*A	88 (28.11)	13 (28.26)	3 (30.00)	5 (20.00)	43 (45.74)	24 (17.39)
*inl*C	85 (27.16)	12 (26.09)	9 (90.00)	6 (24.00)	36 (38.30)	22 (15.94)
*inl*J	131 (41.85)	17 (36.96)	7 (70.00)	18 (72.00)	48 (51.06)	41 (29.71)
*plc*A	53 (16.93)	1 (2.17)	3 (30.00)	3 (12.00)	41 (43.62)	5 (3.623)
*act*A	76 (24.28)	2 (4.35)	5 (50.00)	4 (16.00)	48 (51.06)	17 (12.32)
*hly*A	117 (37.38)	15 (32.6)	5 (50.00)	9 (36.00)	50 (53.19)	38 (27.54)
iap	55 (17.57)	2 (4.35)	1 (10.00)	3 (12.00)	43 (45.74)	6 (4.35)
Total no. of positive isolates	313	46	10	25	94	138

4.3 Antibiotic Resistance Profiles

Among the 313 *L. monocytogenes* serotype-positive isolates, 132 isolates that carried at least three of tested virulence gene markers were chosen to investigate for the profiles of antibiotic resistance. **Table 4.4** summarizes the antibiotic resistance profiles of 132 *L. monocytogenes* isolates against 13 antibiotics from 9 families via disk diffusion method. Over the 132 tested isolates, only 1.51% (2 isolates) was susceptible to all tested antibiotics. Most of the isolates (98.48%) were found to be resistant to oxacillin. In addition, significant levels of resistance were discovered for penicillin (50.00%) and clindamycin (45.45%).

Erythromycin, ciprofloxacin and meropenem were efficient in restraining the growth of *L. monocytogenes* with respective percentage of susceptibility of 90.15%, 88.64%, 87.88%, respectively. **Table 4.5** shows the Multiple Antibiotic Resistance (MAR) Index and resistance pattern of the 132 *L. monocytogenes* towards the 13 antibiotics. Two isolates out of 132 was found to have the highest MAR index value, 0.92, nearly half of the isolates had MAR indices higher than 0.2 (**Figure 4.7**).

Table 4.4: Antibiotic susceptibility profiles of 132 *L. monocytogenes* positive isolates against 13 antibiotics using disc diffusion method.

Antibiotic (Concentration per disc)	Antibiotic susceptibility profile of *L. monocytogenes*		
	Susceptible (%)	Intermediate (%)	Resistant (%)
Beta-lactams			
Amoxicillin/clavulanic acid (AMC, 30 µg)	17 (12.88)	104 (78.79)	11 (8.33)
Oxacillin (OX, 1 µg)	-	2 (1.52)	130 (98.48)
Penicillin (P, 10 µg)	66 (50.00)	-	66 (50.00)
Ampicillin (AMP, 10 µg)	5 (3.79)	105 (79.55)	22 (16.67)
Aminoglycosides			
Gentamicin (CN, 10 µg)	114 (86.36)	-	18 (13.64)
Streptomycin (S, 25 µg)	82 (62.12)	33 (25.00)	17 (12.88)
Macrolides			
Erythromycin (E, 15 µg)	119 (90.15)	7 (5.30)	6 (4.55)
Fluoroquinolones			
Ciprofloxacin (CIP, 5 µg)	117 (88.64)	12 (9.09)	3 (2.27)
Sulfonamides			
Sulfamethoxazole/trimethoprim (SXT, 25 µg)	109 (82.58)	-	23 (17.42)
Rifamycin			
Rifampicin (RD, 5 µg)	70 (53.03)	17 (12.88)	45 (34.09)

Table 4.4 (Continued): Antibiotic susceptibility profiles of 132 *L. monocytogenes* positive isolates against 13 antibiotics using disc diffusion method.

Tetracycline			
Tetracycline (TE, 30 µg)	107 (81.06)	4 (3.03)	21 (15.91)
Carbapenem			
Meropenem (MEM, 10 µg)	116 (87.88)	-	16 (12.12)
Lincosamide			
Clindamycin (DA, 2 µg)	42 (31.82)	30 (22.73)	60 (45.45)

Table 4.5: Multiple Antibiotic Resistance (MAR) Index and resistance pattern of 132 *L. monocytogenes* isolated from both sources.

MAR Index	Antibiotic Resistance Pattern	No. of isolates (%)
0.08	OX	30 (22.73)
0.08	RD	2 (1.52)
0.15	OX/P	19 (14.39)
0.15	OX/AMP	2 (1.52)
0.15	OX/TE	9 (6.82)
0.15	OX/DA	9 (6.82)
0.23	AMC/OX/P	1 (0.76)
0.23	OX/P/AMP	3 (2.27)
0.23	OX/P/DA	3 (2.27)
0.23	OX/RD/DA	11 (8.33)
0.23	OX/P/TE	3 (2.27)
0.23	OX/SXT/RD	1 (0.76)
0.23	OX/TE/DA	1 (0.76)
0.31	OX/P/AMP/DA	2 (1.52)
0.31	OX/P/SXT/MEM	1 (0.76)
0.31	AMC/OX/P/AMP	1 (0.76)
0.31	OX/P/RD/DA	9 (6.82)
0.31	AMC/OX/RD/DA	1 (0.76)
0.38	OX/P/SXT/MEM/DA	1 (0.76)
0.38	AMC/OX/P/AMP/DA	1 (0.76)
0.46	OX/P/AMP/CN/RD/DA	1 (0.76)
0.46	AMC/OX/P/SXT/RD/DA	1 (0.76)
0.46	OX/P/CN/RD/TE/DA	1 (0.76)
0.62	OX/P/CN/S/SXT/RD/MEM/DA	3 (2.27)
0.62	OX/P/AMP/CN/S/SXT/RD/DA	1 (0.76)
0.62	AMC/OX/P/AMP/E/SXT/RD/DA	1 (0.76)
0.69	AMC/OX/P/AMP/E/SXT/RD/TE/DA	1 (0.76)
0.69	OX/P/CN/S/SXT/RD/TE/MEM/DA	3 (2.27)
0.69	OX/P/AMP/CN/S/SXT/RD/MEM/DA	3 (2.27)

Table 4.5 (Continued): Multiple Antibiotic Resistance (MAR) Index and resistance pattern of 132 *L. monocytogenes* isolated from both sources.

0.77	OX/P/AMP/CN/S/SXT/RD/TE/MEM/DA	2 (1.52)
0.77	OX/P/CN/S/E/CIP/SXT/RD/MEM/DA	1 (0.76)
0.77	AMC/OX/P/AMP/CN/S/SXT/RD/MEM/DA	1 (0.76)
0.77	AMC/OX/P/AMP/S/E/SXT/RD/TE/DA	1 (0.76)
0.92	AMC/OX/P/AMP/CN/S/E/CIP/SXT/TE/MEM/DA	1 (0.76)
0.92	AMC/OX/P/AMP/CN/S/E/CIP/SXT/RD/TE/DA	1 (0.76)

AMC – Amoxicillin/clavulanic acid; OX – Oxacillin; P – Penicillin; AMP – Ampicillin; CN – Gentamicin; S – Streptomycin; E – Erythromycin; CIP – Ciprofloxacin; SXT – Sulfamethoxazole/trimethoprim; RD – Rifampicin; TE – Tetracycline; MEM – Meropenem; DA – Clindamycin

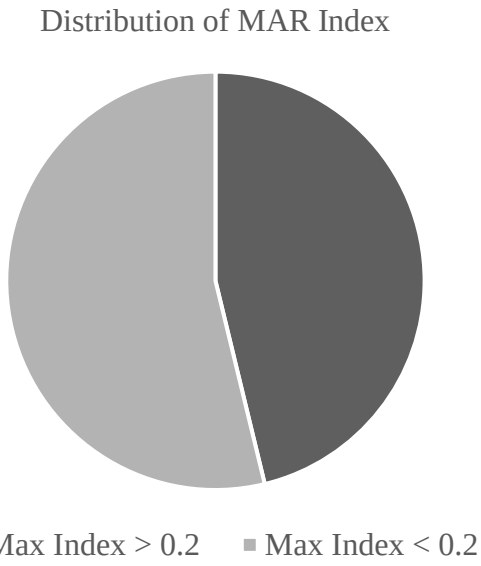
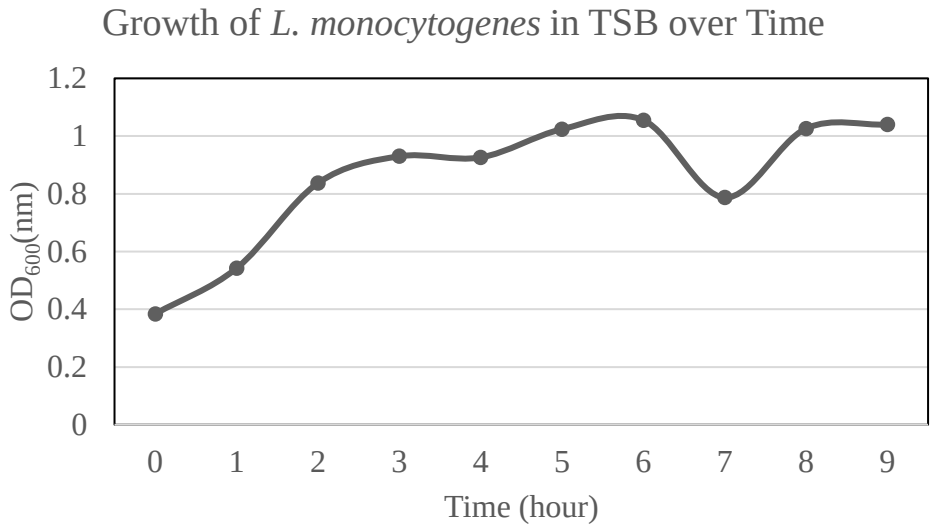


Figure 4.7: Distribution of MAR Index of 132 *L. monocytogenes* positive isolate against 13 antibiotics.

4.4 Biofilm Formation of *L. monocytogenes* in Tryptic Soy Broth (TSB) and Tested Surfaces

The growth of *L. monocytogenes* in TSB was observed over time and the OD value is shown in **Figure 4.8**. The OD value grew in the first five hours and peaked at time point 6 h (OD_{600} of 1.055). The value started to decline at time point 7 h before rising again at 8 h and 9h. Bacteria's growth was in its log phase

between 1 h to 6 h. On the other hand, on various finishing surfaces of stainless-steel coupons, the log number of bacteria was found to steadily increase after 3



hours and reach its highest peak of growth after 6 hours of incubation (#4: 7.91 $\log_{10}$ CFU/cm^2; 2B: 7.68 $\log_{10}$ CFU/cm^2) (**Figure 4.9**). The CFU count decreased between the hours of 6 and 24 and fell to its lowest point during the hours of 24 and 48. The CFU count increased modestly from 48 to 120 hours.

Figure 4.8: Growth curve of *L. monocytogenes* ATCC 19112 in TSB incubated at 37°C from 0 to 9 h were plotted versus represented by OD_{600} (nm).

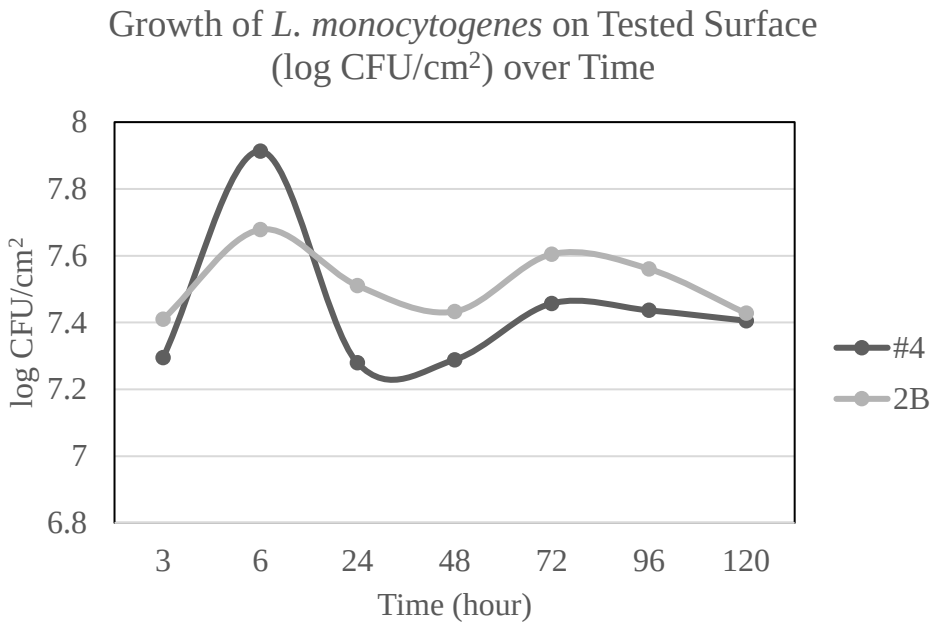


Figure 4.9: Growth curve of *L. monocytogenes* ATCC 19112 on tested surfaces of grade 304 stainless steel no.4 (#4) and 2B at intervals time points of 3 h, 6 h, 24 h, 48 h, 72 h, 96 h and 120 h were plotted against $\log_{10}$ CFU/cm^2.

4.5 Initial Cell Capacity of *L. monocytogenes* in the Biofilm on Test Surfaces

Table 4.6 summarizes the initial cell capacity of biofilm of *L. monocytogenes* of ATCC 19112 and environmental isolate on various test surfaces at respective temperature in $\log_{10}$ CFU/cm^2. Overall, there was a significant difference ($\rho<0.05$) between the cell capacities of ATCC strain and the environmental isolate. The ATCC strain exhibited a slightly higher CFU count than the environmental isolation with estimated marginal means of 8.20±0.21 $\log_{10}$ CFU/cm^2 and 7.81±0.21 $\log_{10}$ CFU/cm^2, respectively.

In addition, there is a significant difference ($\rho<0.05$) in CFU count between the stainless-steel and plastic, Teflon and plastic materials but no significant difference ($\rho>0.05$) between the stainless-steel and Teflon material when comparing based on the same strain but in same or different temperature group. As results showed that there is a substantial difference in the same strain

of *L. monocytogenes* growth on same or different test surfaces at the incubation temperatures of 4°C and 25°C, but not between the 30°C and 37°C. For instance, environmental isolate developed slightly more cells on Teflon at temperatures 37°C (8.33±0.04 log_{10} CFU/cm^2) and 30°C (8.26±0.02 log_{10} CFU/cm^2), than at 4°C (7.92±0.08 log_{10} CFU/cm^2).

Moreover, stainless steel and Teflon exhibited higher cell formation in biofilm than plastic material among three material groups when comparison within same strain but in same or different temperature group. As results revealed that CFU count of ATCC strain on stainless steel (#4) that incubated at 37°C and on Teflon (PTFE) that incubated at 30°C showed closely rates at 8.98±0.13 log_{10} CFU/cm^2 and 8.59±0.13 log_{10} CFU/cm^2, respectively than on plastic (HDPE) that incubated at 30°C at rate of 7.91±0.07 log_{10} CFU/cm^2.

Table 4.6: Enumeration of *L. monocytogenes* strain ATCC 19112 and environmental isolate in biofilm on test surface of stainless-steel, Teflon and plastic material at temperatures of 4°C, 25°C, 30°C and 37°C that expressed in log_{10} CFU/cm^2.

Test Surface	Temp (°C)	ATCC 19112	Environmental Isolate
Stainless-steel			
#4	4	8.01 ± 0.30^{IBa}	7.89 ± 0.06^{IIBa}
	25	8.59 ± 0.27^{IBb}	8.20 ± 0.15^{IIBb}
	30	8.86 ± 0.13^{IBc}	8.21 ± 0.01^{IIBc}
	37	8.98 ± 0.13^{IBc}	7.97 ± 0.46^{IIBc}
2B	4	8.07 ± 0.03^{IBa}	7.87 ± 0.07^{IIBa}
	25	8.55 ± 0.08^{IBb}	8.24 ± 0.11^{IIBb}
	30	8.36 ± 0.22^{IBc}	8.11 ± 0.08^{IIBc}
	37	8.88 ± 0.07^{IBc}	8.30 ± 0.04^{IIBc}
Teflon			
PTFE	4	8.00 ± 0.05^{IBa}	7.92 ± 0.08^{IIBa}
	25	8.54 ± 0.04^{IBb}	8.00 ± 0.28^{IIBb}
	30	8.59 ± 0.13^{IBc}	8.26 ± 0.02^{IIBc}
	37	8.22 ± 0.11^{IBc}	8.33 ± 0.04^{IIBc}

Table 4.6 (Continued): Enumeration of *L. monocytogenes* strain ATCC 19112 and environmental isolate in biofilm on test surface of stainless-steel, Teflon and plastic material at temperatures of 4°C, 25°C, 30°C and 37°C that expressed in log_{10} CFU/cm^2.

Plastic			
HDPE	4	7.47 ± 0.08^{IAa}	7.24 ± 0.08^{IIAa}
	25	7.72 ± 0.11^{IAb}	7.27 ± 0.05^{IIAb}
	30	7.91 ± 0.07^{IAc}	7.32 ± 0.08^{IIAc}
	37	7.68 ± 0.07^{IAc}	7.64 ± 0.20^{IIAc}
PP	4	7.47 ± 0.17^{IAa}	7.39 ± 0.15^{IIAa}
	25	7.50 ± 0.17^{IAb}	7.29 ± 0.03^{IIAb}
	30	8.48 ± 0.39^{IAc}	7.34 ± 0.06^{IIAc}
	37	8.12 ± 0.03^{IAc}	7.47 ± 0.28^{IIAc}

$*^{I-II}$ indicate type of isolate.
$*^{A-B}$ indicate type of test surface.
$*^{a-c}$ indicate incubation temperature of culture.
$*^{I-II}$ Mean±SD within the same row with the same superscripts are not significantly different ($\rho>0.05$) in isolate while data with different superscripts are significantly different ($\rho<0.05$) in isolate.
$*^{A-B}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in test surface while data with different superscripts are significantly different ($\rho<0.05$) in test surface.
$*^{a-c}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in temperature while data with different superscripts are significantly different ($\rho<0.05$) in temperature.

4.6 Viable Cell of *L. monocytogenes* on Test Surfaces After Treatment

L. monocytogenes biofilm's viable cell capacity was assessed after the application of disinfection and sanitizing solutions on to the test surfaces. **Table 4.7** illustrates the viable cell capacity of *L. monocytogenes* on test surfaces after solution treatment. Overall, there is no significant difference with ρ-value of 0.32 (>0.05), between the *L. monocytogenes* ATCC 19112 strain and environmental isolate. After COS PAA (CP) treatment on Teflon (PTFE) surface, the number of viable cells of the ATCC strain and the environmental isolate cultured at 4°C was similarly close, at 5.60 log_{10} CFU/cm^2 and 5.20 log_{10} CFU/cm^2, respectively.

Nevertheless, in terms of culturing temperatures, there was significant differences in the number of viable cells remained on test surface ($\rho<0.05$). The

number of viable ATCC strain cells on #4 stainless-steel surface incubated at 30ºC (7.43 $\log_{10}$ CFU/cm^2) was found to be higher than those incubated at 37ºC (6.88 $\log_{10}$ CFU/cm^2) after the Liquid Sanitizer (LS) treatment.

Additionally, the number of viable cells that persisted on test surfaces varied considerably ($\rho<0.05$) among the three sets of materials, but no significant differences ($\rho>0.05$) was found between the #4 and 2B variations of stainless-steel. In comparison to PTFE surface (6.32 $\log_{10}$ CFU/cm^2), a reduced viable cell capacity of the environmental isolate was found on test surface made of HDPE (4.10 $\log_{10}$ CFU/cm^2), cultured at 4ºC and treated with SS Sanitizer (SS) solution.

In comparison to LS and SS solutions, the CP solution demonstrated higher efficacy to eliminate viable cells developed on test surfaces. After being treated with CP, SS and LS solution, the number of viable environmental isolate cells on 2B stainless-steel surface cultivated at 25ºC was 6.97 $\log_{10}$ CFU/cm^2, 7.07 $\log_{10}$ CFU/cm^2 and 7.13 $\log_{10}$ CFU/cm^2, respectively. The lowest viable cell capacity was observed on the treatment with CP solution.

Table 4.7: Viable cell capacity of biofilms from *L. monocytogenes* ATCC 19112 and environmental isolate after treated with different types of disinfectant and sanitizing solutions on test surface of stainless-steel, Teflon and plastic material at respective temperature of 4ºC, 25ºC, 30ºC and 37ºC and data was expressed in $\log_{10}$ CFU/cm^2.

Test Surface	Temp (ºC)	Treatment	ATCC 19112	Environmental Isolate
Stainless-steel				
#4	4	CP	6.80 ± 0.22^{IDa1}	6.27 ± 0.56^{IDa1}
		LS	6.85 ± 0.05^{IDa3}	6.64 ± 0.61^{IDa3}
		SS	6.58 ± 0.13^{IDa2}	6.84 ± 0.43^{IDa2}
	25	CP	7.17 ± 0.12^{IDb1}	7.10 ± 0.13^{IDb1}
		LS	7.28 ± 0.19^{IDb3}	7.10 ± 0.26^{IDb3}
		SS	7.18 ± 0.06^{IDb2}	7.19 ± 0.07^{IDb2}

Table 4.7 (Continued): Viable cell capacity of biofilms from *L. monocytogenes* ATCC 19112 and environmental isolate after treated with different types of disinfectant and sanitizing solutions on test surface of stainless-steel, Teflon and plastic material at respective temperature of 4°C, 25°C, 30°C and 37°C and data was expressed in log_{10} CFU/cm^2.

	30	CP	7.15 ± 0.16^{IDd1}	7.10 ± 0.11^{IDd1}
		LS	7.43 ± 0.21^{IDd3}	7.23 ± 0.09^{IDd3}
		SS	7.31 ± 0.19^{IDd2}	7.10 ± 0.08^{IDd2}
	37	CP	6.36 ± 0.21^{IDc1}	6.89 ± 0.26^{IDc1}
		LS	6.88 ± 0.12^{IDc3}	7.03 ± 0.07^{IDc3}
		SS	6.99 ± 0.21^{IDc2}	6.89 ± 0.38^{IDc2}
2B	4	CP	6.78 ± 0.20^{IDa1}	6.63 ± 0.36^{IDa1}
		LS	6.87 ± 0.19^{IDa3}	6.91 ± 0.15^{IDa3}
		SS	6.56 ± 0.73^{IDa2}	6.64 ± 0.27^{IDa2}
	25	CP	7.26 ± 0.23^{IDb1}	6.97 ± 0.13^{IDb1}
		LS	7.03 ± 0.33^{IDb3}	7.13 ± 0.17^{IDb3}
		SS	7.13 ± 0.12^{IDb2}	7.07 ± 0.16^{IDb2}
	30	CP	7.21 ± 0.13^{IDd1}	7.13 ± 0.12^{IDd1}
		LS	7.33 ± 0.13^{IDd3}	7.13 ± 0.41^{IDd3}
		SS	7.14 ± 0.12^{IDd2}	7.25 ± 0.16^{IDd2}
	37	CP	6.29 ± 0.38^{IDc1}	7.06 ± 0.14^{IDc1}
		LS	6.95 ± 0.17^{IDc3}	7.02 ± 0.09^{IDc3}
		SS	6.73 ± 0.14^{IDc2}	6.93 ± 0.18^{IDc2}
Teflon				
PTFE	4	CP	5.60 ± 0.88^{ICa1}	5.20 ± 0.65^{ICa1}
		LS	6.67 ± 0.36^{ICa3}	6.81 ± 0.54^{ICa3}
		SS	6.71 ± 0.47^{ICa2}	6.32 ± 0.56^{ICa2}
	25	CP	2.12 ± 0.00^{ICb1}	6.07 ± 0.58^{ICb1}
		LS	6.84 ± 0.76^{ICb3}	7.16 ± 0.30^{ICb3}
		SS	6.98 ± 0.27^{ICb2}	7.23 ± 0.21^{ICb2}
	30	CP	6.04 ± 0.58^{ICd1}	7.18 ± 0.17^{ICd1}
		LS	7.22 ± 0.59^{ICd3}	7.02 ± 0.44^{ICd3}
		SS	6.87 ± 0.36^{ICd2}	7.10 ± 0.24^{ICd2}
	37	CP	6.10 ± 0.83^{ICc1}	6.97 ± 0.43^{ICc1}
		LS	6.69 ± 0.56^{ICc3}	6.48 ± 0.52^{ICc3}
		SS	6.12 ± 0.63^{ICc2}	6.98 ± 0.31^{ICc2}
Plastic				
HDPE	4	CP	4.48 ± 0.38^{IAa1}	5.52 ± 0.48^{IAa1}
		LS	5.70 ± 0.95^{IAa3}	4.66 ± 0.54^{IAa3}
		SS	5.29 ± 0.48^{IAa2}	4.10 ± 0.16^{IAa2}
	25	CP	5.35 ± 0.50^{IAb1}	6.43 ± 0.06^{IAb1}
		LS	5.86 ± 0.41^{IAb3}	5.42 ± 0.61^{IAb3}
		SS	5.61 ± 0.52^{IAb2}	5.13 ± 0.64^{IAb2}
	30	CP	6.41 ± 0.58^{IAd1}	6.49 ± 0.22^{IAd1}
		LS	6.14 ± 0.24^{IAd3}	6.09 ± 0.49^{IAd3}
		SS	6.47 ± 0.44^{IAd2}	5.46 ± 0.47^{IAd2}
	37	CP	6.53 ± 0.25^{IAc1}	6.72 ± 0.16^{IAc1}
		LS	6.13 ± 0.67^{IAc3}	5.89 ± 0.32^{IAc3}
		SS	5.95 ± 0.37^{IAc2}	5.62 ± 0.35^{IAc2}

Table 4.7 (Continued): Viable cell capacity of biofilms from *L. monocytogenes* ATCC 19112 and environmental isolate after treated with different types of disinfectant and sanitizing solutions on test surface of stainless-steel, Teflon and plastic material at respective temperature of 4°C, 25°C, 30°C and 37°C and data was expressed in log_{10} CFU/cm^2.

PP	4	CP	4.81 ± 0.59^{IBa1}	5.54 ± 0.45^{IBa1}
		LS	5.91 ± 0.74^{IBa3}	4.91 ± 0.89^{IBa3}
		SS	4.66 ± 0.74^{IBa2}	4.47 ± 0.52^{IBa2}
	25	CP	5.61 ± 0.59^{IBb1}	6.44 ± 0.10^{IBb1}
		LS	5.93 ± 0.36^{IBb3}	6.05 ± 0.45^{IBb3}
		SS	6.48 ± 0.30^{IBb2}	6.24 ± 0.27^{IBb2}
	30	CP	7.03 ± 0.09^{IBd1}	6.88 ± 0.16^{IBd1}
		LS	6.62 ± 0.26^{IBd3}	5.96 ± 0.26^{IBd3}
		SS	6.57 ± 0.53^{IBd2}	6.14 ± 0.39^{IBd2}
	37	CP	6.88 ± 0.49^{IBc1}	7.03 ± 0.40^{IBc1}
		LS	7.36 ± 0.26^{IBc3}	6.44 ± 0.34^{IBc3}
		SS	6.90 ± 0.52^{IBc2}	6.67 ± 0.26^{IBc2}

*CP indicates disinfectant solution of COS PAA.
*LS indicates sanitizing solution of Liquid Sanitizer.
*SS indicates sanitizing solution of SS Sanitizer.
$*^{I}$ indicates type of isolate.
$*^{A-D}$ indicate type of test surface.
$*^{a-d}$ indicate incubation temperature of culture.
$*^{1-3}$ indicate type of disinfectant and sanitizing solutions.
$*^{I}$ Mean±SD within the same row with the same superscripts are not significantly different ($\rho>0.05$) in isolate.
$*^{A-D}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in test surface while data with different superscripts are significantly different ($\rho<0.05$) in test surface.
$*^{a-d}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in temperature while data with different superscripts are significantly different ($\rho<0.05$) in temperature.
$*^{1-3}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in treatment while data with different superscripts are significantly different ($\rho<0.05$) in treatment.

4.7 Effectiveness of Disinfectant and Sanitizing Solution to treat Biofilm of *L. monocytogenes*

Table 4.8 summarizes the log reduction of cell capacity after treatment in log_{10} CFU/cm^2 and percentage of reduction for both ATCC strain and environmental isolate in different cultivation condition. Overall, the log reduction between the ATCC strain and environmental isolate differs significantly. The estimated

marginal mean value for ATCC strain cell was 1.77±0.22 $\log_{10}$ CFU/cm^2 and indicating a larger log reduction of cell capacity compared to the environmental isolate (1.35±0.22 $\log_{10}$ CFU/cm^2).

Different test surfaces employed in the study had substantial impact on the log reduction rate of *L. monocytogenes* cell capacity in biofilms. The cell reduction that on surface of 2B stainless steel differs significantly from groups of Teflon and plastic. When compared to the cell number of environment strain grown on PTFE (1.12 $\log_{10}$ CFU/cm^2) and HDPE (2.58 $\log_{10}$ CFU/cm^2) at 4ºC after LS treatment, the cell reduction on 2B stainless-steel surface was in 0.97 $\log_{10}$ CFU/cm^2, which was noticeably different. However, there was no significant difference between the cell reduction formed on #4 stainless-steel and PP surfaces. For instance, despite the different application of treatment solution, the log reduction of the cell capacity of an environmental isolate produced on #4 stainless steel at 25ºC was 0.99 $\log_{10}$ CFU/cm^2, which is quite similar to the log reduction of the cell grown on PP surface at (0.85 $\log_{10}$ CFU/cm^2).

In addition, *L. monocytogenes* cell reduction rates in biofilm are greatly influenced by cultivation temperature. Log_{10} reduction of cell capacity that developed at 4ºC exhibits a larger mean value of reduction when compared to cells grown at 30ºC. The reduction rate of ATCC strain on HDPE surface at 4ºC was found to be 40.05% (2.99 $\log_{10}$ CFU/cm^2), which was significantly higher than ATCC strain on HDPE surface at 30ºC (18.99%, 1.50 $\log_{10}$ CFU/cm^2) after treated with CP solution.

The application of CP solution to the biofilm of *L. monocytogenes* grown at 25ºC demonstrated a highly significant cell reduction rate of 75.17% (6.42 $\log_{10}$ CFU/cm^2) in ATCC strain on PTFE surface when compared to application

of LS and SS treatment, which yielded reduction rates of 19.85% (1.70 log_{10} CFU/cm^2) and 18.19% (1.55 log_{10} CFU/cm^2), respectively.

Table 4.8: Log_{10} reduction of cell capacity of biofilm from *L. monocytogenes* of strain ATCC 19112 and environmental isolate after treated with different types of disinfectant and sanitizing solutions on test surface of stainless-steel, Teflon and plastic material at respective temperature of 4°C, 25°C, 30°C and 37°C and analysis was expressed in log_{10} CFU/cm^2 and percentage reduction (%).

Test Surface	Temp (°C)	Treat-ment	ATCC 19112		Environmental Isolate	
			Log_{10} CFU/cm^2	(%)	Log_{10} CFU/cm^2	(%)
Stainless-steel						
#4	4	CP	1.21 ± 0.24^{IABc3}	15.06	1.63 ± 0.51^{IIABc3}	20.64
		LS	1.16 ± 0.28^{IABc1}	14.44	1.25 ± 0.58^{IIABc1}	15.86
		SS	1.43 ± 0.30^{IABc2}	17.82	1.05 ± 0.46^{IIABcb2}	13.28
	25	CP	1.42 ± 0.34^{IABb3}	16.45	1.09 ± 0.07^{IIABb3}	13.34
		LS	1.32 ± 0.22^{IABb1}	15.27	1.09 ± 0.35^{IIABb1}	13.24
		SS	1.41 ± 0.23^{IABb2}	16.38	1.01 ± 0.13^{IIABb2}	12.29
	30	CP	1.71 ± 0.08^{IABa3}	19.30	1.11 ± 0.11^{IIABa3}	13.52
		LS	1.43 ± 0.23^{IABa1}	16.15	0.99 ± 0.09^{IIABa1}	11.99
		SS	1.55 ± 0.27^{IABa2}	17.48	1.11 ± 0.08^{IIABa2}	13.56
	37	CP	2.62 ± 0.27^{IABb3}	29.12	1.08 ± 0.58^{IIABb3}	13.22
		LS	2.10 ± 0.20^{IABb1}	23.33	0.94 ± 0.39^{IIABb1}	11.57
		SS	1.99 ± 0.28^{IABb2}	22.16	1.08 ± 0.33^{IIABb2}	13.52
2B	4	CP	1.31 ± 0.21^{IAc3}	16.15	1.24 ± 0.35^{IIAc3}	15.86
		LS	1.22 ± 0.20^{IAc1}	15.06	0.97 ± 0.14^{IIAc1}	12.30
		SS	1.53 ± 0.74^{IAc2}	18.87	1.24 ± 0.30^{IIAc2}	15.71
	25	CP	1.29 ± 0.19^{IAb3}	15.14	1.27 ± 0.21^{IIAb3}	15.41
		LS	1.52 ± 0.29^{IAb1}	17.75	1.11 ± 0.23^{IIAb1}	13.49
		SS	1.42 ± 0.14^{IAb2}	16.64	1.17 ± 0.17^{IIAb2}	14.19
	30	CP	1.15 ± 0.28^{IAa3}	13.72	0.99 ± 0.12^{IIAa3}	12.18
		LS	1.03 ± 0.18^{IAa1}	12.29	0.98 ± 0.45^{IIAa1}	12.05
		SS	1.22 ± 0.24^{IAa2}	14.57	0.87 ± 0.19^{IIAa2}	10.65
	37	CP	2.59 ± 0.36^{IAb3}	29.15	1.23 ± 0.14^{IIAb3}	14.87
		LS	1.92 ± 0.16^{IAb1}	21.67	1.27 ± 0.07^{IIAb1}	15.29
		SS	2.15 ± 0.14^{IAb2}	24.24	1.37 ± 0.16^{IIAb2}	16.54

Table 4.8 (Continued): Log_{10} reduction of cell capacity of biofilm from *L. monocytogenes* of strain ATCC 19112 and environmental isolate after treated with different types of disinfectant and sanitizing solutions on test surface of stainless-steel, Teflon and plastic material at respective temperature of 4°C, 25°C, 30°C and 37°C and analysis was expressed in log_{10} CFU/cm^2 and percentage reduction (%).

Teflon						
PTFE	4	CP	2.10 ± 0.85^{ICc3}	30.01	2.73 ± 0.66^{IICc3}	34.43
		LS	1.33 ± 0.32^{ICc1}	16.60	1.12 ± 0.51^{IICc1}	14.14
		SS	1.29 ± 0.46^{ICc2}	16.11	1.61 ± 0.51^{IICc2}	20.34
	25	CP	6.42 ± 0.03^{ICb3}	75.17	1.92 ± 0.65^{IICb3}	23.93
		LS	1.70 ± 0.77^{ICb1}	19.85	0.84 ± 0.32^{IICb1}	10.42
		SS	1.55 ± 0.28^{ICb2}	18.19	0.77 ± 0.41^{IICb2}	9.45
	30	CP	2.56 ± 0.62^{ICa3}	29.70	1.09 ± 0.17^{IICa3}	13.13
		LS	1.37 ± 0.62^{ICa1}	15.91	1.24 ± 0.45^{IICa1}	15.02
		SS	1.72 ± 0.36^{ICa2}	20.01	1.16 ± 0.25^{IICa2}	14.03
	37	CP	2.12 ± 0.80^{ICb3}	25.78	1.36 ± 0.40^{IICb3}	16.37
		LS	1.54 ± 0.48^{ICb1}	18.72	1.85 ± 0.51^{IICb1}	22.20
		SS	2.10 ± 0.70^{ICb2}	25.48	1.35 ± 0.31^{IICb2}	16.18
Plastic						
HDPE	4	CP	2.99 ± 0.33^{ICc3}	40.05	1.72 ± 0.44^{IICc3}	23.74
		LS	1.77 ± 0.95^{ICc1}	23.66	2.58 ± 0.52^{IICc1}	35.68
		SS	2.18 ± 0.41^{ICc2}	29.26	3.14 ± 0.13^{IICc2}	43.38
	25	CP	2.37 ± 0.45^{ICb3}	30.74	0.84 ± 0.07^{IICb3}	11.56
		LS	1.86 ± 0.47^{ICb1}	24.09	1.85 ± 0.62^{IICb1}	25.48
		SS	2.10 ± 0.51^{ICb2}	27.26	2.14 ± 0.62^{IICb2}	29.50
	30	CP	1.50 ± 0.59^{ICa3}	18.99	0.83 ± 0.22^{IICa3}	11.32
		LS	1.78 ± 0.29^{ICa1}	22.42	1.22 ± 0.45^{IICa1}	16.75
		SS	1.45 ± 0.44^{ICa2}	18.26	1.86 ± 0.42^{IICa2}	25.43
	37	CP	1.15 ± 0.28^{ICb3}	14.97	0.92 ± 0.15^{IICb3}	12.05
		LS	1.56 ± 0.68^{ICb1}	20.26	1.75 ± 0.39^{IICb1}	22.86
		SS	1.74 ± 0.36^{ICb2}	22.59	2.02 ± 0.44^{IICb2}	26.40
PP	4	CP	2.66 ± 0.60^{IBc3}	35.57	1.85 ± 0.40^{IIBc3}	25.01
		LS	1.56 ± 0.70^{IBc1}	20.89	2.48 ± 0.89^{IIBc1}	33.52
		SS	2.81 ± 0.76^{IBc2}	37.59	2.92 ± 0.48^{IIBc2}	39.57
	25	CP	1.89 ± 0.63^{IBb3}	25.14	0.85 ± 0.12^{IIBb3}	11.70
		LS	1.57 ± 0.31^{IBb1}	20.98	1.25 ± 0.47^{IIBb1}	17.07
		SS	1.02 ± 0.31^{IBb2}	13.56	1.05 ± 0.27^{IIBb2}	14.45
	30	CP	1.45 ± 0.34^{IBa3}	17.03	0.47 ± 0.13^{IIBa3}	6.36
		LS	1.86 ± 0.45^{IBa1}	21.80	1.39 ± 0.25^{IIBa1}	18.91
		SS	1.91 ± 0.58^{IBa2}	22.41	1.20 ± 0.39^{IIBa2}	16.38

Table 4.8 (Continued): Log_{10} reduction of cell capacity of biofilm from *L. monocytogenes* of strain ATCC 19112 and environmental isolate after treated with different types of disinfectant and sanitizing solutions on test surface of stainless-steel, Teflon and plastic material at respective temperature of 4°C, 25°C, 30°C and 37°C and analysis was expressed in log_{10} CFU/cm^2 and percentage reduction (%).

37	CP	1.24 ± 0.48^{IBb3}	15.28	0.47 ± 0.40^{IIBb3}	6.20
	LS	0.76 ± 0.28^{IBb1}	9.32	1.03 ± 0.26^{IIBb1}	13.78
	SS	1.22 ± 0.52^{IBb2}	15.05	0.80 ± 0.31^{IIBb2}	10.59

*CP indicates disinfectant solution of COS PAA.
*LS indicates sanitizing solution of Liquid Sanitizer.
*SS indicates sanitizing solution of SS Sanitizer.
$*^{I-II}$ indicate type of isolate.
$*^{A-D}$ indicate type of test surface.
$*^{a-b}$ indicate incubation temperature of culture.
$*^{1-3}$ indicate type of disinfectant and sanitizing solutions.
$*^{I-II}$ Mean±SD within the same row with the same superscripts are not significantly different ($\rho>0.05$) in isolate while data with different superscripts are significantly different ($\rho<0.05$) in isolate.
$*^{A-D}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in test surface while data with different superscripts are significantly different ($\rho<0.05$) in test surface.
$*^{a-b}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in temperature while data with different superscripts are significantly different ($\rho<0.05$) in temperature.
$*^{1-3}$ Mean±SD within the same column with the same superscripts are not significantly different ($\rho>0.05$) in treatment while data with different superscripts are significantly different ($\rho<0.05$) in treatment.

4.8 Scanning Electron Microscopy (SEM)

Figure 4.10 illustrates the initial attachment of *L. monocytogenes* ATCC 19112 strain on #4 stainless steel surface for temperatures of 4ºC, 25ºC, 30ºC and 37ºC at magnification of 2000x. Despite the temperature changes, *L. monocytogenes* was shown to have a high cellular density in four test coupons. Each test coupon revealed cells with honeycomb structure that were uniformly attached. Among the four test coupons, test coupon (B) had the highest cellular density.

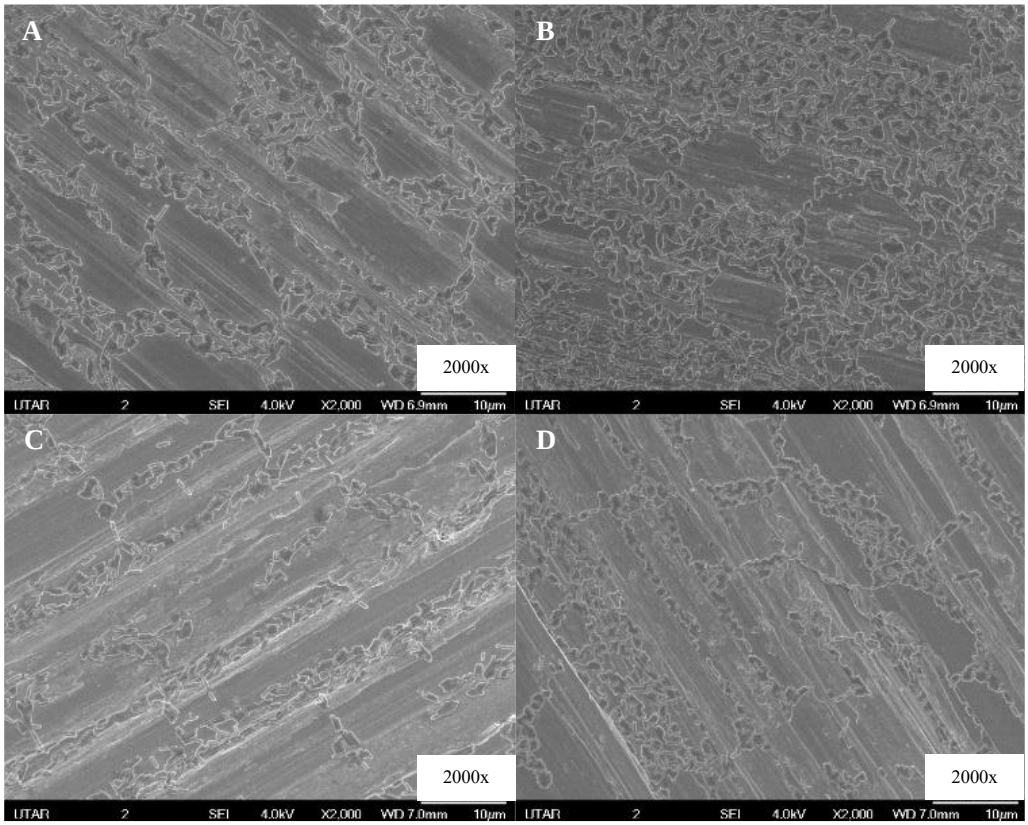


Figure 4.10: SEM images at magnification of 2000x showing initial attachment of *L. monocytogenes* ATCC 19112 strain on stainless steel no. 4 finishing line (#4) on different temperatures: (A) 37ºC, (B) 30ºC, (C) 25ºC and (D) 4ºC.

Figure 4.11 demonstrates the initial attachment of *L. monocytogenes* strain of ATCC 19112 on #4 stainless steel surface for different temperatures of 4ºC, 25ºC, 30ºC and 37ºC at magnification of 5000x. Four test coupons from (E) to (H) showed the evidence of the development of EPS (extracellular polymeric substances) matrix. Planktonic cells were gathered up into the form of microcolony which observed in test coupons of (E) and (F). Elongated cells were observed in test coupon of (G) and (H). Test coupon (H) showed some abnormal small size of cells due to the low temperature of 4ºC.

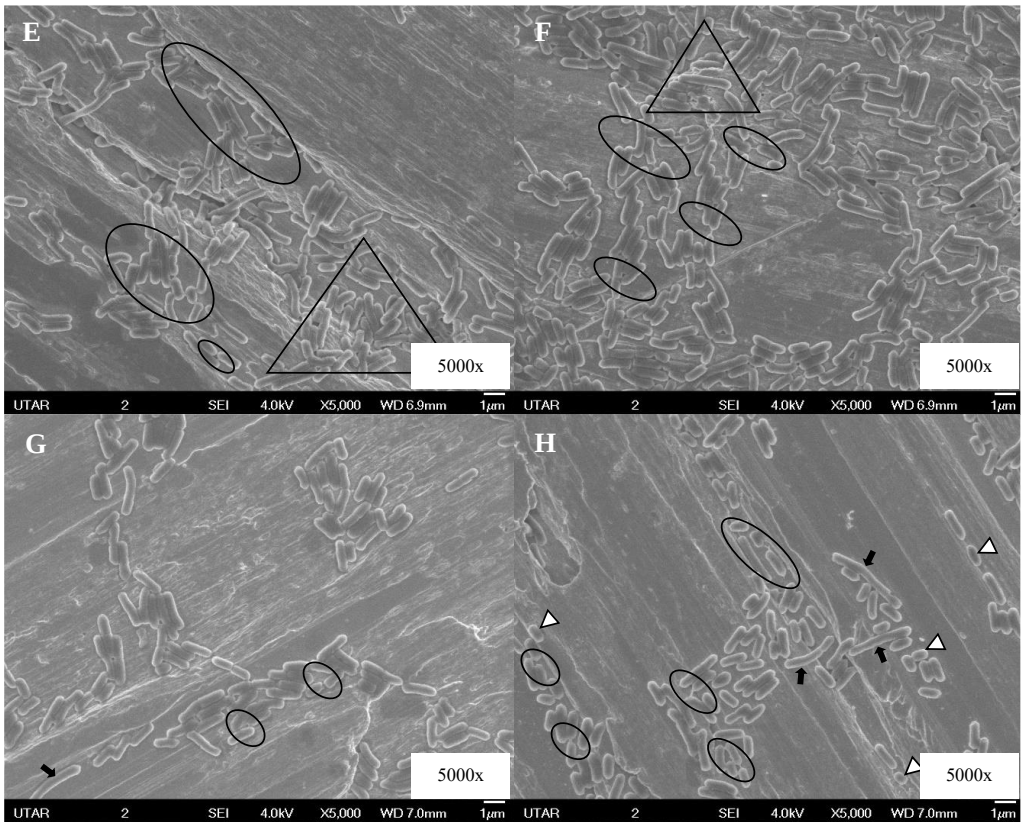


Figure 4.11: SEM images in the magnification of 5000x showing initial attachment of *L. monocytogenes* ATCC 19112 strain on stainless steel no. 4 finishing line (#4) on different temperatures: (E) 37ºC, (F) 30ºC, (G) 25ºC and (H) 4ºC. EPS are marked in oval-shaped line; microcolony are highlighted with triangle-shaped line; black arrows denote elongated cells and white filled triangle symbols indicate abnormal small size of cells.

Figure 4.12 illustrates the final attachment of *L. monocytogenes* ATCC 19112 strain on #4 stainless steel surface on different temperatures 4ºC, 25ºC, 30ºC and 37ºC at magnification of 10000x after the treatment with CP solution. With CP treatment, some cells suffered damages: shrinking and wrinkles morphologies were observed (denoted by curved-rectangle line). After receiving CP treatment, some injured cells displayed crevices on their exterior surfaces. However, the generated EPS matrix remained unchanged after the treatment.

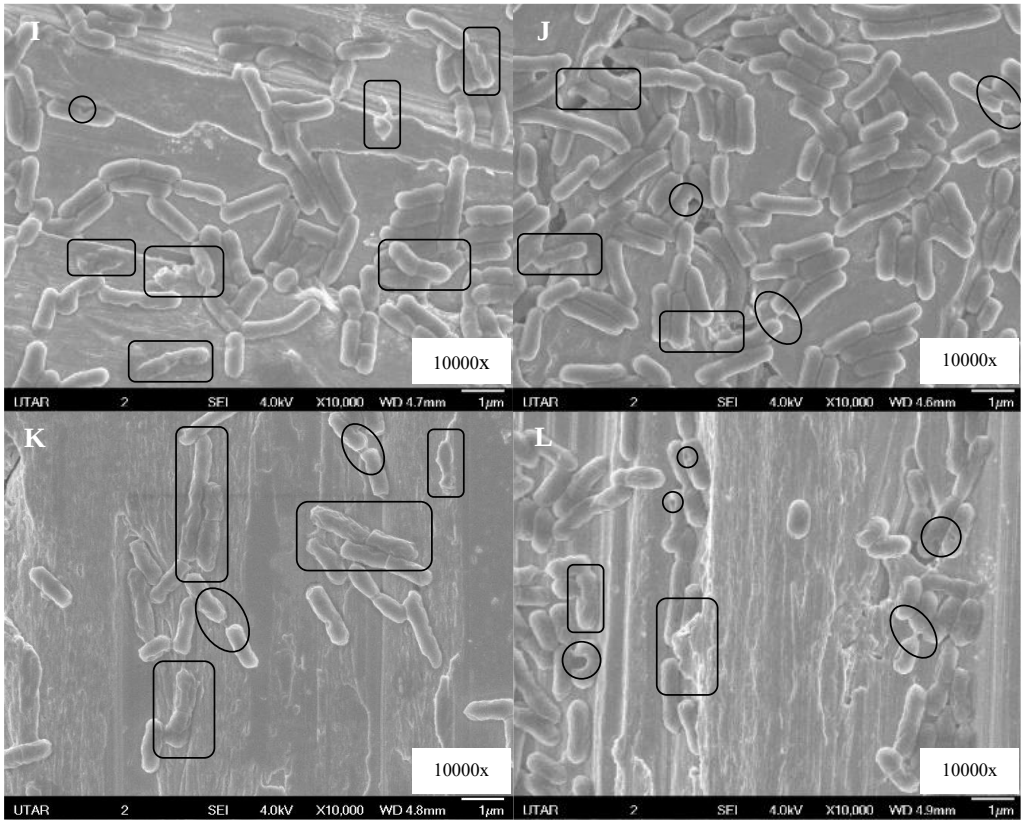


Figure 4.12: SEM images in the magnification of 10000x showing final attachment of *L. monocytogenes* ATCC 19112 strain on stainless steel no. 4 finishing line (#4) on different temperatures after treated with CP solution: (I) 37ºC, (J) 30ºC, (K) 25 ºC and (L) 4ºC. EPS are marked in oval-shaped line; curved-rectangle line denote injured cells and cells in crevice are marked in circle-shaped line.

Figure 4.13 shows the initial attachment of *L. monocytogenes* strain of environmental isolate on #4 stainless steel surface on different temperatures of 4ºC, 25ºC, 30ºC and 37ºC at magnification of 2000x. In comparison to **Figure 4.10**, four test coupons seen in **Figure 4.13** had a reduced cellular density of *L. monocytogenes*. Even with a low cell density, cells on each test coupon were equally interconnected in a honeycomb structure. In **Figure 4.13**, the lowest cellular density was found in test coupon (P).

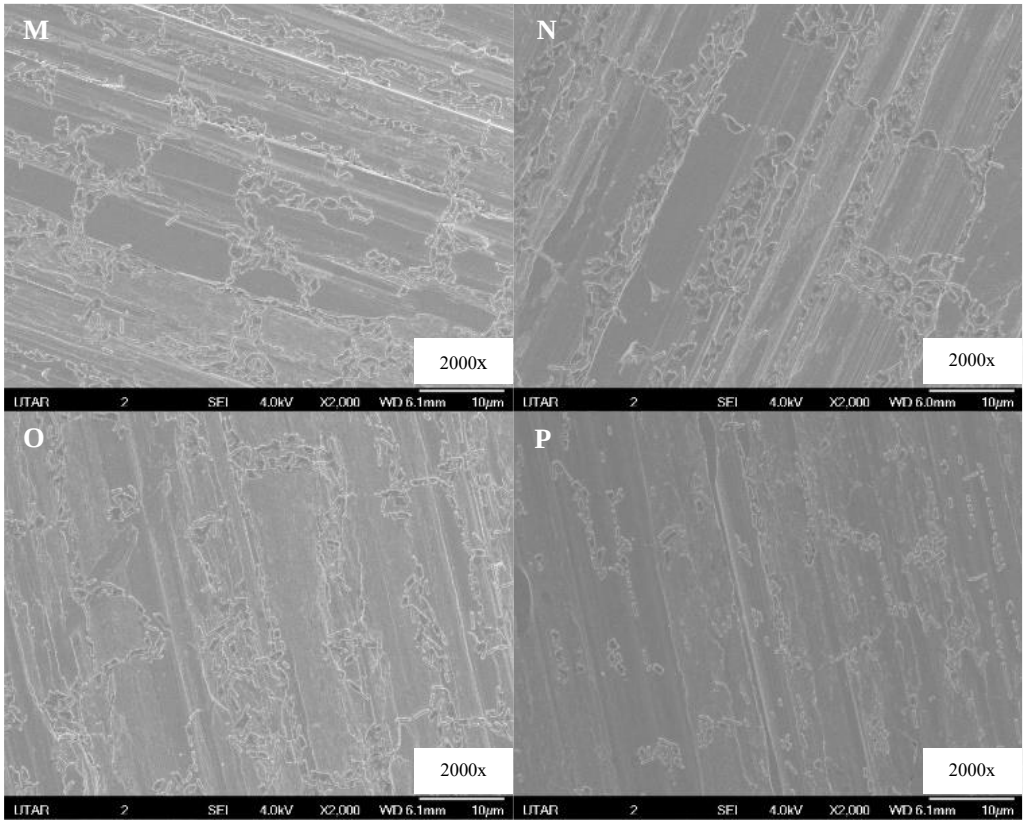


Figure 4.13: SEM images in the magnification of 2000x showing initial attachment of *L. monocytogenes* strain of environmental isolate on stainless steel no. 4 finishing line (#4) on different temperatures: (M) 37ºC, (N) 30ºC, (O) 25ºC and (P) 4ºC.

Figure 4.14 illustrates the initial attachment of *L. monocytogenes* strain of environmental isolate on #4 stainless steel surface on different temperatures 4ºC, 25ºC, 30ºC and 37ºC at magnification of 5000x. Overall, it was discovered that less EPS matrix was produced than in **Figure 4.11**. In addition, the growth of EPS matrix was lesser from test coupons (Q) to (T), which corresponded to the incubation temperature. Formation of microcolonies were observed in test coupon of (Q). Test coupon (T) was the only one displayed the growth of elongated cells. Abnormally small cells were visible in coupon (T), which was grown at low temperature of 4ºC.

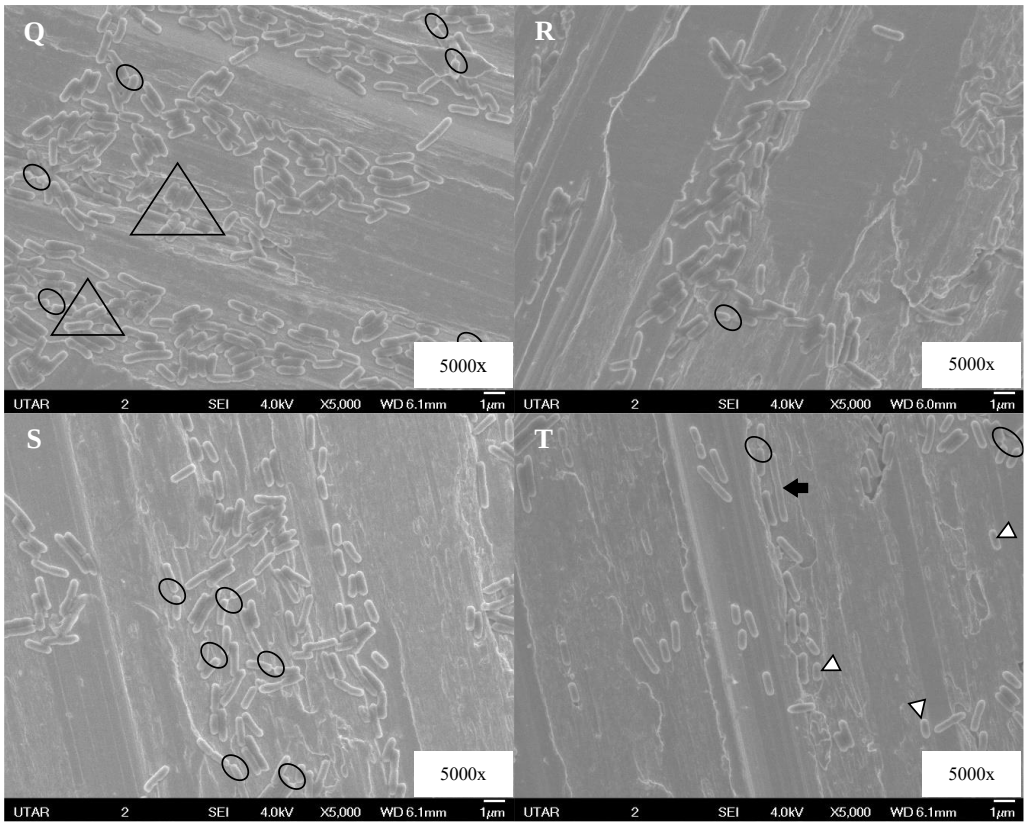


Figure 4.14: SEM images in the magnification of 5000x showing initial attachment of *L. monocytogenes* strain of environmental isolate on stainless steel no. 4 finishing line (#4) on different temperatures: (Q) 37ºC (R) 30ºC, (S) 25ºC and (T) 4ºC. EPS are marked in oval-shaped line; microcolony are highlighted with triangle-shaped line; black arrows denote elongated cells and white filled triangle symbols indicate abnormal small size of cells.

Figure 4.15 shows the initial attachment of *L. monocytogenes* strain of environmental isolate on #4 stainless steel surface on different temperatures of 4ºC, 25ºC, 30ºC and 37ºC at magnification of 10000x after treated with CP solution. Upon CP treatment, some cells experienced injury from cuts and some were lysed into fragments (denoted by curved-rectangle line). Some damaged cells showed holes on their outermost surfaces after getting CP treatment. Apart from of test coupon (X), the generated EPS matrix was left intact.

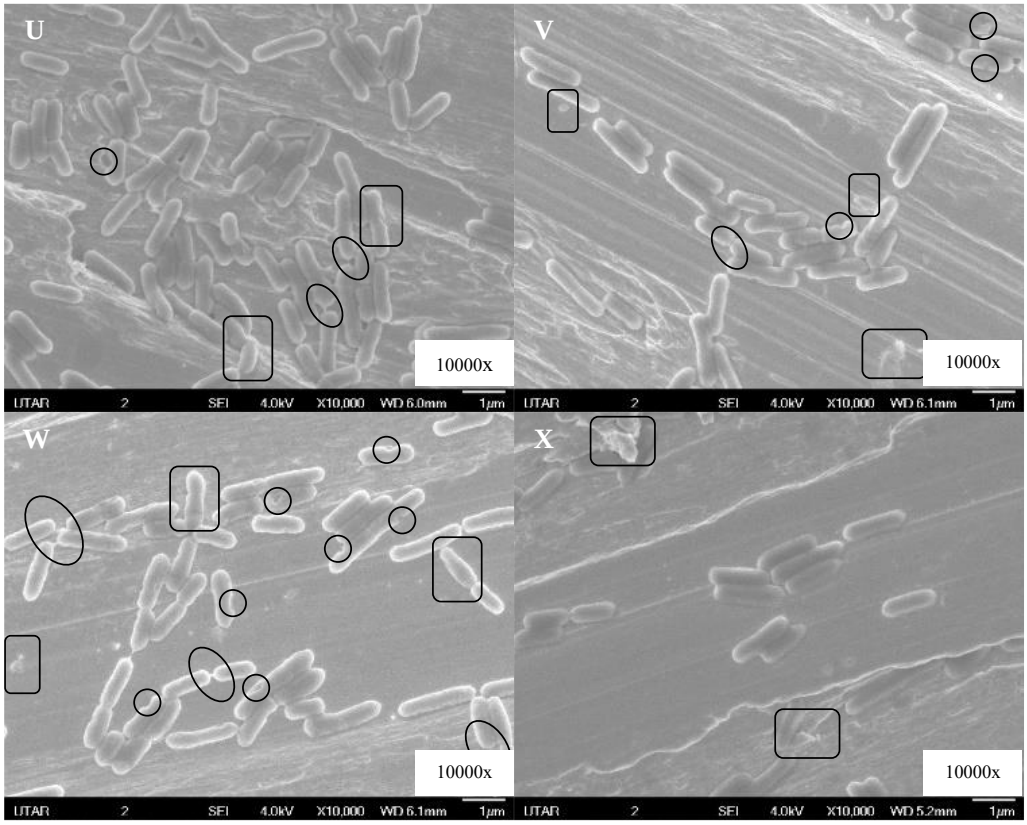


Figure 4.15: SEM images in the magnification of 10000x showing final attachment of *L. monocytogenes* strain of environmental isolate on stainless steel no. 4 finishing line (#4) on different temperatures after treated with CP solution: (U) 37ºC, (V) 30ºC, (W) 25ºC and (X) 4ºC. EPS are marked in oval-shaped line; curved-rectangle line denote injured cells and cells in crevice are marked in circle-shaped line.

CHAPTER 5

DISCUSSION

5.1 Prevalence of *Listeria* in food and food contact surfaces

Listeriosis, a potentially fatal foodborne illness, can be brought on by consuming food contaminated with *L. monocytogenes*. The origin of the contamination is the subject of the queries. It is postulated that bacteria were exposed at the food processing facility and began to grow during storage and at the retail level. The results of this present study prove that *Listeria* contamination can emerge on both sources of food and food contact surfaces. Based on the **Table 4.1**, the prevalence of *Listeria* spp. and *L. monocytogenes* in food sources were 28.82% (n= 49/170) and 15.29% (n=26/170) respectively. On the other hand, there were 13.16% (n=20/152) and 9.87% (n=15/152), respectively, of *Listeria* spp. and *L. monocytogenes* in food contact surface. It was found that the prevalence of *Listeria* spp. and *L. monocytogenes* varied statistically between the both sources as food were likely to be contaminated by *L. monocytogenes* compared to food contact surfaces.

This finding is in line with that of Leong et al. (2014), who found a high significant prevalence of *L. monocytogenes* in food samples. This might be as a result of the fact that food samples have favourable intrinsic characteristics relating to nutritional content, water activity, and pH levels that naturally promote the growth of *L. monocytogenes*, which are lacking on food contact surfaces (Center for Food Safety and Applied Nutrition, 2017; Rolfe and Daryaei,

2020). Additionally, consuming raw food like salad, steak tartare, and sushi is growing increasingly popular in various nations throughout the world. This could possibly be attributed to the reality that food samples, as opposed to food contact surfaces, are more likely to be possible source of *L. monocytogenes* contamination. As a result, the prevalence count of *Listeria* spp. and *L. monocytogenes* in food sources are relatively high than in food contact surfaces.

The food category with the highest prevalence of *L. monocytogenes* among the four listed in **Table 3.1** was processed food (33.33%), followed by minimally processed food (31.25%), raw food (26.32%) and ready-to-eat (RTE) food (4.26%). It indicates that processed food is likely to be contaminated among the four food categories. Among the collected processed food samples, processed meat such as chicken slice and smoked duck thigh (n=2/6, 33.33%) found to be more likely contaminated by *L. monocytogenes*. This observation was consistent with the study conducted by Wong et al. (2012) who had discovered that 22.33% of burger patties tested positive for *L. monocytogenes*. Similarly, Marian et al, (2012) had reported that 33.3% of the burger samples collected from local wet markets, mini markets and supermarkets in Selangor, Malaysia was contaminated with *L. monocytogenes*.

This result may be explained by the fact that lengthy processing steps required for processed food seemed to increase the likelihood of contamination. One or a combination of various processes, such as washing, chopping, pasteurizing, freezing, fermenting, packaging, and cooking are carried out before turning fresh food into food products (USDA Agricultural Marketing Service, 2008). Fresh food able to contact with various surfaces before turned into processed food. However, despite the harsh processing method adopted, *L.*

monocytogenes has the capability to strive for survival. Moreover, processed food is substituted with salts, fats, sugar, spices, preservatives and additives for flavour and texture. According to Webster et al. (2009) and Mhurchu et al. (2010) processed meat were the food categories with the highest amount of salt content. Salt added to processed food able to reduce water activity in food and limit the growth of pathogens (Doyle and Glass, 2010). Yet this is not applicable to control the proliferation *L. monocytogenes*. Due to the characteristic of salt tolerant, *L. monocytogenes* able to grow for at least 20 hours in low moisture with high osmotic strength environments such as highly saturated (40% v/v) salt solution (Tapia de Daza et al, 1991; Bayles and Wilkinson, 2000; Liu et al. (2005). Thus, addition of salt may serve as effective enrichment agent for the growth *L. monocytogenes* in processed food.

In addition, cryotolerance characteristics of *L. monocytogenes*, it may proliferate slowly under cold environment. Since most processed food is stored at low temperature to preserve its freshness, this may generate a climate that is conducive to bacterial growth. Besides that, processed food typically requires to be defrosted before cooking. If processed food that has been infected with *Listeria* is defrosted, recontamination will occur during the thawing process. *L. monocytogenes* will be liberated and begin to multiply as the ambient temperature rises throughout the thawing process (Freezing Technology, 2011). *L. monocytogenes* is not only cryotolerant but also freeze-thaw tolerant Azizoglu et al. (2009). Additionally, Kataoka et al. (2017) had discovered that *L. monocytogenes* was still capable of reacting and proliferating with increment of 2 $\log_{10}$ CFU/g in frozen corn, green peas, crabmeat, and shrimp at temperature range of 4 to 20 even after 7 days of storage in freezer. Consequently, frozen

processed food products are a potential reservoir for the growth of *L. monocytogenes*.

Besides processed food, RTE food was discovered to consist high levels of sodium, sugar and saturated fat (Poti et al., 2016). It goes through a similar processing stages as processed food does. However, no further cooking or preparation required for RTE before consumption. It is more convenient than processed food product in preparation, which simply require to be heated through thermal processing (hot water, steam, microwave or infrared) before being served (Huang and Hwang, 2012). Some RTE foods, such fresh salads with leafy vegetables or sliced raw meats, sandwiches, cheeses and others, are even prepared to be served cold or room temperature without any heat to cook the food (Huang and Hwang., 2012; Adnan et al., 2021). Thus, RTE food likely to act as a transport vehicle to transmit *L. monocytogenes* to consumers.

In the present study, *L. monocytogenes* was detected in 4.26% (n=4/93) of RTE food samples derived from fruits (n=1/12, 8.33%), sandwiches (n=1/13, 7.69%), desserts (n=1/25, 4%) and cooked food (n=1/27, 3.7%). Surprisingly, the prevalence of RTE food was found to be the lowest prevalence count among four food categories and did not show significant difference between processed food in present study. The prevalence count was determined to be substantially lower than in the earlier study that was reported. For instance, Ponniah et al. (2010) had reported that 22.5% of RTE vegetables were positive for *L. monocytogenes*. Also, studies by Jamali et al. (2013), Leong et al. (2014) and Mureddu et al. (2014) had reported the presence of *L. monocytogenes* in RTE food samples at a rate ranging from 5.0 to 30.0%. Due to proper packaging and temperature control, the majority of the evaluated RTE food samples were

individually packaged and shown on an enclosed display shelf at 4ºC for fresh salad boxes, desserts, sushi rolls and sandwiches, 25ºC for cooked food and 60ºC for hot food in a steaming rack. This resulted in a low prevalence count in present study. RTE food is able to maintain its freshness and reduce the likelihood of contamination it reaches the consumer at retail level.

On the other hand, minimally processed food is another reservoir for *L. monocytogenes* to proliferate in food samples. It was discovered statistically differ from processed and RTE foods in the present study. *L. monocytogenes* was found in pre-cut vegetables (n=5/18, 27.7%), pre-cut meats (n=2/5, 40%), pre-cut fishes (n=2/6, 33.33%) and bean curds (n=1/2, 50%) at rate of 31.25% (n=10/33) The findings are in line with research carried out in Turkey and Japan. Researchers from these two places tested *L. monocytogenes* positive in ground beef (7.2%), minced beef (12.2%) and chicken meat (17.8%) samples from the retail premises (Inoue et al., 2000; Kalender, 2011). According to the food classification tool (NOVA classification) developed by Monteiro et al. (2016), minimally processed food is a natural food product that goes through mild processing without the addition of flavouring, salt, and sugar. Poultry, meat, seafood, steaks, fillets, fresh-cut fruits, and vegetables, as well as fresh or dried herbs such as mint and thyme, are included in this category.

Fresh produce is then subjected to minimal processing, including sorting, washing, peeling, slicing, chopping, grinding, and eliminating any inedible components (Bansal et al. 2015). With the utilization of machineries for mild processing, such as grinder surfaces and blades, could be contaminated with *L. monocytogenes* due to the ideal acclimatization of the production environment and insufficient cleaning of the intricate structure of the machinery. This can be

explained by the discovery of *L. monocytogenes* contamination in pre-cut meats, fish, poultry and vegetables in the present study. In addition, mild processed food products usually display refrigerated display rack to prolong its shelf life before reaching to the consumer. If *L. monocytogenes* was developed during the processing procedures, long term preservation in refrigeration at 4^{o}C provides an optimal growth environment for *L. monocytogenes* to proliferate (Swaminathan et al., 2007). As a psychrotrophic pathogen, *L. monocytogenes* able absorb glycine, betaine and carnitine from food and utilize them as cryoprotectants, to survive and thrive in low temperatures for extended period of time (Bayles and Wilkinson, 2000).

However, there is no statistically significant difference between minimally processed food and raw food in present study. Both food categories required additional preparation before consumption as they were retained in the natural state or undergo minimal processing before being distributed and reaching consumers. Raw food originates from natural or unprocessed ingredients that are obtained from both plant and animal sources and sourced directly from farms (Poti et al., 2015; Monteiro at al., 2016). Faeces, wastewater and soil can contaminate raw food, for instance, polluted soil affects the majority of plant-based raw food sources. Due to the utilization of fertilizers as a substitute for other soil amendments to increase crop growth and production. However, compared to soil treated with natural fertilizers, soil treated with chemical fertilizers promotes a growing environment that is favourable for *Listeria* development (Szymczack et al., 2014). Additionally, improper wastewater treatment procedures utilized at farms can also lead to cross-contamination (Lyautey et al., 2007). Contaminated wastewater will be

transferred from the plantation's upper to lower end and trapped in the soil able persisted for up to 84 days (Vivant et al, 2013). In the end, there may be more instances of cross-contamination with harvested crops across the entire plantation site. Therefore, it is conceivable for *L. monocytogenes* to induce cross-contamination in fresh vegetables like bok choy, cabbages, Chinese chives, potatoes, tomatoes, and bean sprouts whether acquired from wet market or hypermarket, as determined in the current study.

Furthermore, it was discovered that *L. monocytogenes* can easily contaminate raw food from animal sources. According to Osaili et al. (2010), *Listeria* spp. and *L. monocytogenes* were present in meat and poultry products in Jordan at rates of 1.8% to 48% and 2.7% to 20%, respectively. This is due to several factors of cross contamination during slaughtering, improper hygienic practices and improper handling practices (holding temperature). In addition, the animal itself constituted a second source of infection. According to Fredriksson-Ahomaa et al., (2009), 50 slaughter pigs in South Germany were discovered to be carrying *L. monocytogenes*, which was primarily located in tonsillar tissue (32%) and part of which was found in pig faeces (4%). Surprisingly, the present study found no raw animal food sources that were contaminated with *L. monocytogenes*. Despite the fact that the prevalence of *L. monocytogenes* in the raw food category was less than 30% in present study, the presence of this foodborne pathogen in 10 out of 38 samples raised concerns about the risk of listeriosis. In order to minimize the risk of acquiring listeriosis by decreasing the possibility of contamination of raw food before consumption, it is advised to wash produce well and heat it to safe cooking temperature, ideally at 74ºC for poultry, 71ºC for ground meat, 58ºC for vegetables (Van, 2020, Caroline, 2023).

Listeria eradication is extremely difficult since it can attempt to survive in hostile environments. In order to prevent the infection from spreading, it is crucial to identify *L. monocytogenes* in food processing plants and food contact surfaces before it penetrates into the end products. In the present study, *Listeria* spp. was detected on the direct food contact surfaces (n=14/93, 15.05%) of conveyor belts, working benches for food processing and preparation, as well as on the surfaces of cutting boards. However, the highest prevalence of *L. monocytogenes* in direct food contact surfaces (n=11/93, 11.83%) was detected mainly from food processing equipment such as cooling machine, orientation cup, and roller bar. Our results indicated that poor cleaning and a cooling system that operates at a low temperature, *L. monocytogenes* is able to thrive and persist in cooling machine, especially particularly at the edges of the machine which supplemented by food debris for its growth. Food residue accumulating near the machine's edge encourages the growth of biofilm-forming *L. monocytogenes* on food processing machinery. The persistence of *L. monocytogenes* biofilm development was demonstrated by Carpentier and Cerf (2011) and Hoelzer et al. (2011) in the US, who revealed that *L. monocytogenes* was still present on in the refrigerated food processing surfaces and equipment after routine washing and disinfecting. This also accords with our earlier observations, which once again demonstrated the remarkable adaptability of *L. monocytogenes* in hostile environments.

Additionally, the present study discovered the presence of *L. monocytogenes* in complicated machinery used to convey food products throughout the manufacturing process, such as orientation cups and roller bars. However, it is challenging to disassemble for mid-shift cleaning and may

adversely influence the quality and safety of food product. This finding supports evidence from previous observations by Schäfer et al. (2017) revealed that *Listeria* spp. contamination occurred at several stages of the chicken fractionation process in the poultry plant with initial contamination rates of 33.3% (bleeding, plucking and evisceration), 50% (after evisceration) and 76.2% (packing). This proved that there was cross-contamination throughout the fractionation procedure, and it was as a result of the automated fractionation machine's design, which made it difficult to maintain cleanliness (Schäfer et al., 2017). Thus, the propagation of *L. monocytogenes* will occur through the unclean sections. Moreover, implementation of intermittent sanitization practises towards complicated machinery in food processing plants constituted a significant risk of *L. monocytogenes* contamination (Lundén et al., 2003). Thus, application of GMP (Good Manufacturing Practices) and HACCP (Hazards Analysis Critical Control Points) monitoring system are suggested to both visited food processing plant in Perak, Malaysia to ensure the quality and safety of finished product. Besides that, it is advised to put into practise the Seek & Destroy approach as well, which is essential to control and restrict development niches that may have existed in processing plants for a long time and consequently cross-contaminated the finished food product (Butts, 2003).

Contrary to predictions, the present study did not discover a statistically significant difference between direct and indirect food contact surfaces. The most obvious finding to emerge from the analysis is that *L. monocytogenes* contamination in food processing plant and retail food premises can occur on direct and indirect food contact surfaces. Based on the results in **Table 4.1**, *Listeria* spp. and *L. monocytogenes* were detected in an area of stainless-steel

racks to hold trays used in food preparation, stainless-steel racks to hold baskets used in processing lines and cleaning cloths used in retail premises at rate of 10.17% (n=6/59), 6.78% (n=4/59), respectively. This was observed in the study by Leong et al. (2014), who discovered that *L. monocytogenes* was prevalent in 4.4% of environmental samples collected from 48 food business operators in the Republic of Ireland which processing dairy, meat, seafood, fresh-cut vegetables and other food sectors. However, the rate of *L. monocytogenes contamination* on non-direct food contact surfaces, such as floors, walls, drying rooms and steaming rooms in meat processing lines, was relatively higher than the previous studies in Leong et al. (2014), ranging from 11.0% to 25.0% (Thévenot et al., 2005; Mureddu et al., 2014). Thus, the potential threat of cross-contamination must be noted in any food processing plants, regardless of whether the rate of contamination is high or low.

The findings might be explained by the introduction of *L. monocytogenes* into the processing line via incoming raw materials that entry with trolleys that may get in contact with the underneath floor and drains (Cutter, 2017). Since the production line is still dry at this point, *L. monocytogenes* is still not reproducing effectively (Lakicevic and Nastasijevic, 2016; Cutter, 2017). However, researchers have reported that mid-shift sanitation around food processing areas with water under high pressure spraying for minimal of 2 seconds can lead to the transmission of *L. monocytogenes* through the air (Ministry of Agriculture and Forestry, 2011; Berrang, et al., 2013). The exposure of water to clean the processing area has intensively created a favourable habitat (a cold and wet atmosphere with food residue) for *L. monocytogenes* to reside and further result in biofilm forms (Lakicevic and Nastasijevic, 2016). The detection of *L.*

monocytogenes on stainless-steel racks utilized in the processing line in the present study, which are often omitted during sanitation and may pre-contaminated before or during production.

In addition, the unhygienic practices of food handlers may be a factor promoting the development of listeriosis. A high workload in every shift raises the risk of contamination if food handlers do not distinguish between clean and dirty areas and reduces the frequency of cleaning in the food processing area (Schäfer et al., 2017; Mpundu et al., 2022). Additionally, poor personnel hygiene practises of food handlers, such as improper hand washing after using the lavatory (Food and Drug Administration, 2012; Cutter, 2017). This is due to previous studies shown that *L. monocytogenes* is present in 1-10% of healthy human faeces (Sauders, et al., 2005; Harfner, 2021). Thus, it is possible to spread via dirty hands and subsequently cross-contaminate the processing environment and food product. Besides that, customers may carry *L. monocytogenes* on their hands, shoes and clothing during their visit to the retail store for shopping. Retail workers may pick up this pathogen during routine work activities via cross-contamination routes, and it may then transfer to food or cleaning tools. This is demonstrated by the detection of *L. monocytogenes* on cleaning cloths that collected in the study from food processing plants and retail premises. As a result, retail workers are more likely to get infected by those in processing plants to cross contaminate with *L. monocytogenes* as most of them work in an environment without personal protective equipment (PPE), which can function as a shield to reduce the rate of contact for workers in food processing plant. Therefore, it is essential to educate the food handlers the importance of personnel hygienic practices and safe food handling techniques through courses. With the

implementation of proper sanitation procedures in regular basis, it is likely to reduce the incidence of sporadic cases of listeriosis from the sector of food processing plants and retail food premises towards the consumers.

On the other hand, the overall prevalence rates of *Listeria* spp. and *L. monocytogenes* from food and food contact surfaces in the present study were 21.43% (n=69/322) and 12.73% (n=41/322), respectively. Although the prevalence in both sources is less than 50%, it seems like not detrimental to the study but poses a risk of listeriosis incidence in Perak, Malaysia, as large number of samples from various sources and categories were collected for analysis. Since the United States, Australia, Austria, New Zealand, and Italy have a zero-tolerance policy towards *L. monocytogenes* in 25g of food, while in European countries food has a tolerance of less than 100 cfu/g for *L. monocytogenes* contamination. But both standards are accepted in Canada and Denmark (Food and Agriculture Organization of the United Nations 1999; EFSA, 2013).

However, currently has no laws or regulations that address the control of *Listeria* spp. and *L. monocytogenes* contamination of food and food products in Malaysia. Nevertheless, between 1994 and 2013, researchers discovered that *L. monocytogenes* was present in a wide variety of foods at prevalence rates that were highly varied, including 60.0% in chicken portions, 75.0% in frozen beef, 24.1% in chicken meat, 22.5% in vegetables, 33.33% in frozen chicken burger patties, 42.03% in chicken breast, 14.7% in ready-to-eat food, 26.39% in chicken offal, 33.33% in beef offal (Arumugaswamy et al., 1994; Hassan et al., 2001; Lihan and Samuel, 2007; Ponniah et al., 2010; Wong et al., 2012; Goh et al., 2012; Jamali, 2013; Kuan et al., 2013a, Kuan et al., 2013b). Despite this, no research has been done on the environment of food and food products are

processed in Malaysia, where the food processing environment is one of the paths to drive *L. monocytogenes* contamination from fresh produce to finished food product. However, our findings imply that *L. monocytogenes* contamination was not limited to the food and food products; it was also found in the food processing environment in Perak, Malaysia. This contamination needs to be further investigated in other states throughout Malaysia to obtain comprehensive data or information that can accurately represent the entire country of Malaysia. In the light of this, it may be essential to create laws regarding *L. monocytogenes* contamination in order to limit or lower the rate of contamination before circumstances worsen and markedly increased likelihood of human listeriosis manifesting in the future arises.

5.2 Classification of *L. monocytogenes*

5.2.1 Serotype

Due to its ability to cause listeriosis, a total of 351 *L. monocytogenes* isolates that collected from positive samples in food sources and on food contact surfaces were then studied about their serotyping group. Out of 351 isolates, only 313 isolates able to recover from the stock from the previous analysis. There are several possible explanations for this observation: a) extracted DNA was being prepared in the prevalence study analysis and being degraded along the storage time as it no longer sequestered in the cell under favorable growth condition as environmental before being isolated; b) extracted DNA was being isolated from environmental sources is challenging as extracted DNA may not stable as it may reduce in DNA fragment size or consisted lesions that blocked the DNA replication further leading to unretrievable DNA for the study (Dabney et al.,

2013). Thus, degraded isolates (n=23) were not able recovered in the analysis and were excluded from the data analysis.

Among the 14 serotypes, serotype of 1/2a, 1/2b and 4b are commonly associated (approximately 95%) in human listeriosis clinical cases (Kathariou, 2002; Orsi et al., 2011; Wu et al., 2015). Serotype 1/2a mainly predominates in contaminated food while serotype 4b contributed for the overwhelming of cases in human listeriosis outbreak (Vasconcelos et al., 2008). However, in the present study, serogroup III (4a-4c) showed the highest prevalence of 44.09% (138/313), followed by serogroup II.2 (1/2b-3b-7) at 30.03% (94/313), serogroup I.1 (1/2a-3a) at 14.70% (46/313), serogroup II.1 (4b-4d-4e) at 7.99% (25/313) and serogroup I.2 (1/2-3c) at 3.19% (10/313). Surprisingly, serogroup III showed the highest prevalence count for serotyping which was found in the samples collected from sources of food and food contact surfaces in the present study. Despite the fact that it was previously rarely found implicated in human listeriosis and determined to be pathogenic to animals but not humans (Chen et al., 2009a; Orsi et al., 2011).

However, the unexpected results in the present study have highlighted that serogroup III (also belongs to lineage III and IV) may be a novel serogroup within *L. monocytogenes* other than serogroup I and II, which are frequently associated with human listeriosis. Furthermore, it was revealed to have a higher variety in phenotypic and genetic diversity when comparing it to strains from serogroups I and II (Liu et al., 2006; Roberts et al., 2006). To date, the subdivision of this serogroups is still limited only discovered sub-serogroups of IIIA-1, IIIA-2, IIIB and IIIC which including serotypes 4a, 4c and atypical 4b (Liu et al., 2006; Roberts et al., 2006; Zhao et al., 2011). In 2006, researchers

had discovered that three sub-serogroups of serogroup III (IIIA, IIIIB and IIIC) have the potential to cause human listeriosis as being isolated from human clinical cases whereby some carried virulence gene *Ima*A and some without to cause pathogenicity but majority are cytopathogenic in cell culture plaque assay (Roberts et al., 2006). In 2009, researchers found that serogroup III were the evolutionary of intermediates between *L. monocytogenes* and *L. innocua* but had minimal pathogenicity due to altered *act*A and *plc*B virulence genes (Chen et al., 2009b). In 2011, researchers reported that, in addition to serogroup IIIA-2, others sub-serogroups showed virulence levels comparable to serogroups I and II (Zhao et al., 2011). Thus, it is crucial to note that serogroup III may convey some pathogenic genes from *L. monocytogenes* to human via food or cross-contamination in processing plant, especially the discovery of evolution of this subdivision till date still poorly understood.

In addition to most prevalent non-pathogenic serogroup III, approximately one-third of detected isolates (n=119/313, 38.02%) belonged to the pathogenic serogroup II.1 and II.2. Both serogroups II.1 and II.2 belong to the group of lineage I. Serogroup II.2 showed a higher prevalence in the present study compared to serogroup II.1 and mainly isolated in both sources (food and food contact surfaces), especially in raw food (n=20/57, 35.09%) and indirect food contact surfaces (n=15/28, 53.57%). These results are in accordance with the incidence of serotype 1/2b (lineage I) 36.8% in raw poultry meat and 100% soft cheese reported by Vitas et al. (2004) at retail in Spain, and with 89% of detection of serotype 1/2b (lineage I) isolates obtained by López et al. (2008) from carcasses and non-contact sites from the environment of broiler abattoir in Spain. Yet, serogroup II.1 was primarily isolated from RTE (food source) at

20.31% (n=13/64). Additionally, results obtained in the present study which serogroup II.1 is in line with the previous studies by Vitas and Garcia-Jalon (2004) and Cheng, et al. (2022).

There are notable variations between serogroup II.1 and II.2 despite two serogroups are belonging to lineage I and closely aligned in genomic level. Serogroup II.2 (serotype 1/2b strains) are more likely detected in environmental samples rather than food samples. This can be proven by the studies conducted by Haubert et al. (2015). Lakicevic et al. (2021) reported that serotype 1/2b was averagely colder resistant compared to serotype 4b, thus it was highly possibilities detected in food processing environment as the environment in processing line always keep in the low temperature condition to keep the freshness of food products. However, serogroup II.1 (serotype 4b strain) found to be better adapted in human host than food and food processing environment as they were most likely isolated from human clinical cases via ingestion of *L. monocytogenes* contaminated food (Kathariou, 2002). This does not, however, imply that serotype 4b did not establish themselves in food and food processing environment. Furthermore, this is demonstrated the discovery of serotype 4b (serogroup II.1, lineage I) in the present study. This is due to serotype 4b strains are more sensitive to selective enrichment protocols than other serotypes, hence resulting the presence of serotype 4b strains in food and food processing environments are being underestimated or not allow a true representation in the prevalence count (Kathariou, 2002; Bruhn et al., 2005). With that, there is a need to invent a new isolation protocol for a better monitor and detect serotype 4b strains in food and food processing environment.

Moreover, serogroup I.1 (from lineage II) also being detected in the present study at incidence level of 14.70% (46/313). It was mainly derived from the processed food (n=16/65, 24.62%) and direct food contact surfaces (n=2/8, 25%). In contrast to the previous studies, the present study identified less serogroup I.1 in samples from food and food processing environment. According to the previous studies, serotype 1/2a was predominated in Italy at retail from meat and cheese products and in China at retail from variety of food with rates of 84.7% and 47.9%, respectively (Iannetti et al., 2016; Zhang et al., 2019). Low incidence of serogroup I.1 than previous studies may be due to the variation in time, sample categories and geographical distribution (Ranjbar and Halaji, 2018). On the other hand, serogroup I.2 had the lowest prevalence count of serotyping in the present study at rate 3.19% (10/313). It was mainly isolated from RTE food (n=7/64, 10.94%), mild-processed food (n=2/91, 2.20%) and processed food (n=1/65, 1.54%). The results are in line with the finding that carried out in Republic of Ireland, whereby 15% out of 370 isolates from food and food processing environment were found to be 1/2c serotype (Leong et al., 2014). Braga et al. (2017) also revealed that low incidence of isolation from 1 out of 44 frozen food samples at 4.17% in Uruguay.

According to the earlier findings of Doumith et al (2004a) and Vasconcelos et al. (2008), both serogroups of lineage II share similarities as both of them were intermediate pathogenic potential and typically isolated from contaminated food and food environments associated with the sporadic cases of *L. monocytogenes* not human listeriosis outbreak cases, especially serotype 1/2c in serogroup I.2. Serotype 1/2c is an unusual and and rare serotype that has been identified in human clinical (listeriosis outbreak) cases (Pontello et al., 2012;

Gelbíčova et al., 2016). However, serotype 1/2a in serogroup I.1 does not share the similar finding as it was found predominant isolates from varieties of food categories in recent years in France, United States, Turkey, China and as well as Italy (Boscher et al., 2012; Haley et al., 2015; Kevenk and Gulel, 2015; Wang et al., 2017). Hence it may be displacing serotype 4b as the dominant serotypes other than serotype 1/2b (Ranjbar and Halaji, 2018).

On the other hand, based on the results in **Table 4.2**, lineage I (n=119/313, 38.02%) has a greater recovery rate than lineage II (n=56/313, 17.89%), which increases the likelihood that they would be the source of an outbreak human listeriosis cases. In the comparison to lineage II strains, lineage I strains were found to be more salt tolerant, receptive to alkaline stress and more resistant to oxidative damage. With the presence of stress-related genes such as SSI-1 or SSI-2 and other plasmids carrying genes associated to stress conditions of heavy metals or biocides, lineage I strains of *L. monocytogenes* are better able to persist under harsh circumstances (Lakicevic et al., 2021). Additionally, SSI-1 stress genes had a broader spectrum of adaption than SSI-2 which allowed the strains to survive in food and increase their pathogenicity in humans, ultimately causing listeriosis (Lakicevic et al., 2021). In addition, researchers' team of Muchaamba (2022) revealed that lineage I strains, particularly serotype 4b strains, were the most virulent compared to lineage II strains. In the zebrafish embryo-based infection model that carried out by Muchaamba et al. (2022), serotype 4b strains are capable of 100% death rate at 24-48 hpi. As a result, it can be inferred that given the high prevalence of lineage I strains found in present study, consumers and food handlers in Perak state are probably exposed to potential hazards of *L. monocytogenes* infection.

There are several limitations in the present study as only performed molecular serotyping on the collected isolates and was unable to differentiate between the serovars 1/2a, 1/2c, 4b, 1/2b, 4a and 4c from their respective serogroups (Doumith et al., 2004b). In addition, genomic DNA of pathogen may mutate, lead to phenotypic shifts and resulting in poor discriminatory power in differentiating serovar levels (Nadon et al., 2001; Londero et al., 2019). As a result, optimization may be required to obtain reliable results. In order to solve the aforementioned matters, pulse-field gel electrophoresis (PFGE) typing techniques utilizing restriction enzymes of *Asc*I and *Apa*I are suggested to be carried out after first line screening with molecular serotyping (Borucki et al., 2004; Londero et al., 2019). Since PFGE technique is sensitive to discriminate between serovar levels among strains for the diagnosis of listeriosis outbreaks with high discriminatory power and exhibited stable and reproducible data despite the cost in terms of time, labor and environment for analysis (Londero et al., 2019). Other than PFGE technique, enzyme-linked immunosorbent assay (ELISA) conduct together with commercial kit of *L. monocytogenes* antisera is alternative serotyping technique which is low cost, efficient and widely accessible in both clinical and research laboratories (Palumbo et al., 2003). Hence, a combination of molecular and restriction enzyme linked PFGE techniques or ELISA assay serotyping with commercial kit technique is recommended to conduct in order to study about the epidemiology of *L. monocytogenes* and effectively monitor, control and reduce the possibilities of the occurrence of human listeriosis in Perak or Malaysia.

5.2.2 Virulence Genes

Virulence genes determination is just as important as serotype categorization as virulence gene may contributes the virulent factor of each isolated strain. Virulent factor of 313 serotyped isolates were then determined by using virulent gene markers of *inl*A, *inl*C, *inl*J, *plc*A, *act*A, *hly*A and *iap*. The majority of the 313 serotyped isolates (n=131/313, 41.85%) were determined to predominately contain the internalin gene of *inl*J, as described in **Table 4.3**. This finding showed that 131 isolates were highly virulent to host whereby directly facilitating passage across the intestinal barrier and subsequently causing listeriosis infection. Out of 131 isolates, 48 isolates belonged to serogroup of II.2 and the LPXTG protein-encoding gene (*inl*J) was present in more than half of serogroup II.2 positive isolates (n=48/94, 51.06%). In addition to serogroup II.2, over 72% (n=18/25) of serogroup II.1 isolates and 70% (n=7/10) of serogroup I.2 isolates also harboured the internalin J (*inl*J) virulence gene marker. Other than internalin J (*inl*J), there were other internalin group of virulence factors were being detected in serotyped isolates. These included internalin A (*inl*A), which was discovered in 88 isolates (n=88/313, 28.11%) and internalin C (*inl*C), which was discovered in 85 isolates (n=85/313, 27.16%), as tabulated in **Table 4.3**. Similar to internalin J (*inl*J), internalin A (*inl*A) (n=43/88, 48.86%) and internalin C (*inl*C) (n=36/85, 42.35%) were also primarily found in the serogroup II.2. Surprisingly, internalin C (*inl*C) was discovered to make up about 90% (n=9/10) of serogroup I.2.

These observations from the current investigation provide compelling evidence that human listeriosis outbreaks are commonly associated with serogroups of I.2, II.1 and II.2. As internalin A (*inl*A) is a species-specific surface protein linked with virulence that is crucial for listerial entrance into the host

whereas internalin C (*inl*C) is a gene marker that involved in the virulence throughout the post intestinal phases of *L. monocytogenes* infection in host (Liu et al., 2007). As a result, after the host has been infected via oral ingestion, *L. monocytogenes* will enter the host via the assistance of internalin A (*inl*A), further spreading the pathogenicity to the host's intestinal tract with the support of internalin J (*inl*J) and further boosting up the virulence with gene marker of internalin C (*inl*C) at the post intestinal stages so that it able to proliferate throughout the host to lengthen their life in the infected host (Sabet et al., 2005; Liu et al., 2007).

After internalin J (*inl*J), hemolysin A (*hly*A) was discovered to be the second most predominant virulence factor (n=117/313, 37.38%) as shown in **Table 4.3**. As it able to encode for listeriolysin O (LLO) which is the main virulent factor of *L. monocytogenes* (Kathariou, 2002). Surprisingly, the prevalent count of the *hly*A gene in the present study is lower than expected and disagrees with the findings of Jallewar et al. (2007) stated that practically all isolates of *L. monocytogenes* carried the *hly*A gene. Yet it was only discovered in 117 isolates in the present study. This might due to the mutation or evolution of DNA fragment in the strain profiles by changing the sequence of protein homology of gene hly*A* in the collected isolates of *L. monocytogenes* (Soni and Dubey, 2014; Osman et al., 2020). Similar observations were obtained in the previous studies conducted by Ndahi et al. (2013), stated that only one out of 12 *L. monocytogenes* isolates from raw meat and meat products carried *hly*A gene and Al-Nabulsi et al. (2015a) reported that no *hly*A gene was detected in any of the 66 *L. monocytogenes* isolates from raw and processed meat samples.

Besides the 3 internalin group virulence gene factors stated previously, virulence gene marker, *act*A was found in 76 isolates out 313 serotyped isolates (24.28%), followed by *iap* gene (n=55/313, 17.57%) and lastly *plc*A gene (n=53/313, 16.93%), as described in **Table 4.3**. These virulence gene markers have a relatively low prevalence than internalin related virulence genes. Since act*A* gene was determined not to be a ubiquitous virulence factor in *L. monocytogenes* as it was being triggered by the secretion of virulence factor by gene *inl*C to stimulate the activity of cell to cell spread (Du et al., 2017; Quereda et al., 2021). Other than *act*A gene, the present study revealed low levels of the *iap* gene in *L. monocytogenes* that associated with invasion in the host cell. This resulted in low levels of p60 protein production which limiting the cell viability of the host cell to fight against bacteriolytic activity (Wuenscher et al., 1993). In addition, due to the lack of the virulence gene of *hly*A in some isolates, which is associated with the inability of LLO activity and further resulting in the absence of the complementary activity (action of phospholipase) that is required for the lysis of phagocytic vacuoles to release pathogenic toxin for replication and thus *plc*A gene was found to be less common in the present study (Paramithiotis et al., 2021, Quereda et al., 2021).

Although the overall prevalence of virulence genes of *act*A, *iap* and *plc*A were lower compared to internalin related virulence genes in *L. monocytogenes*, results showed that the majority of isolates were belonged to serogroup II.2, whereby 53.19% (n=50/94) consisted of *hly*A gene, 51.06% (n=48/94) for the *act*A gene, 45.74% (n=43/94) for the *iap* gene and 43.62% (n=41/94) for the *plc*A gene. Results in present study are in line with the findings by Du et al. (2017) and Pyz-Łukasik et al. (2022). These results indicated that isolates from

serogroup II.2 not only possess the internalin-related virulence gene marker, but also possess the involvement of other key virulence-associated proteins and their corresponding genes, such as *act*A, *iap* and *plc*A in order to spread through the host cell after being infected by *L. monocytogenes*.

Among the 313 serotyped isolates, the most tested virulence gene factors were primarily found in isolates from the serogroups of I.2, II.1 and II.2 and thus causing them hypervirulent than other serogroups. These results provide evidence in support of the classification of serotypes observation. It can be concluded that the presence of these serogroups with these virulence gene markers of *L. monocytogenes* in food and food processing environment may contribute to the development of listeriosis. However, less virulence gene markers were found in isolates that belonged to the most prevalent serogroup (serogroup III) in serotyped categorization analysis. According to Liu et al. (2007) most of the isolates that classified under serogroup III was mostly avirulent *L. monocytogenes*.

There are certain analytical constraints in the current investigation, despite the fact that multiplex PCR enables it easier to identify the identity and virulence factor of *L. monocytogenes* with specific virulence gene markers. Firstly, the *prf*A gene, a key protein that involved in the activation of *L. monocytogenes* pathogenicity determinants, is not included in the present study. As *prf*A gene marker is a master regulator or promoter for the virulence genes of *hly*, *mpl* and *act*A in *L. monocytogenes*, it is recommended to add it to future studies in order to address these constraints (Osman et al., 2020). Additionally, researchers found when the *iap* gene was control by *prf*A promoter gene, p60 synthesis in *L. monocytogenes* was reduced and restricting murein hydrolase

activity for septum separation in the host (Bubert et al., 1997). Thus, knowing that the presence of *prf*A gene is crucial to *L. monocytogenes*' pathogenicity in the host before leading to severe human listeriosis outbreaks in the future. Secondly, the absence of *hly*A gene in some of the serotyped isolates due to DNA fragment breakdown along the storage or mutation. Due to the fact that the DNA of collected samples were isolated and extracted from environmental sources, it may bind to dissolved organic matter in the environment to maintain stability and prolong shelf life, even it has been severely degraded and lost some of the targeted DNA fragment when it was being derived from the initial community states in macro-organisms (Toshiaki, 2023). Thus, it was advised to conduct whole genome sequencing in future research in order to understand the mutation or which DNA fragment was altered that was responsible for inability of the targeted gene *hly*A to function.

5.3 Antibiotic Resistance Profiles

In order to control the infection cases in human listeriosis, antibiotics are prescribed to treat *L. monocytogenes* infected patients. Therefore, antibiotic resistance profiles of *L. monocytogenes* towards the common antibiotics that used in treatment were studied. Antibiotic resistance profiles of tested 132 *L. monocytogenes* isolates were summarized in **Table 4.4.** All tested isolates were resistant to at least one antibiotic, however, only 1.51% (2 isolates) was susceptible to all tested antibiotics. In the present study, most of the isolates (98.48%) were found to be resistant to oxacillin, followed by penicillin (50.00%) and clindamycin (45.45%). The result was in agreement with the previous study by Pesavento et al. (2010) reported that *Listeria* spp. that isolated from raw food

and retail foods were found resistant towards oxacillin at the percentage of 75%. While in Spain, Escolar et al. (2017) also found that *Listeria* spp. from animal origin RTE food were resistant against clindamycin (100%), penicillin (32%) and ampicillin (20%). However, in contrast to the finding of the present study, previous studies reported that ampicillin and penicillin were susceptible towards *L. monocytogenes* that isolated from red meat and poultry in Morocco and retail raw food in China, respectively (Ennaji et al., 2008; Wu et al., 2015). Thus, according to the findings in the present study, it is notable that antibacterial agents from beta lactams and aminoglycosides (first line antibiotics or primary choices on treatment) are no longer effective to treat patient infected by listeriosis as reported in previous studies.

On the other hand, erythromycin, ciprofloxacin and meropenem were efficient in constraining the growth of *L. monocytogenes* with respective percentage of susceptibility of 90.15%, 88.64%, 87.88%, respectively, as shown in **Table 4.4**. According to the results in **Table 4.4**, it revealed that antibiotics that originated from macrolides, fluoroquinolones, carbapenem and sulfonamides are effectively against listeriosis. These antibiotic families' groups are commonly considered as the second line antibiotics treatment for listeriosis. These antibiotics will be prescribed to listeriosis infected patients if first line drugs are not working efficiently in the treatment. Results were in agreement with the previous studies (Chin et al., 2018; Andriyanov et al., 2021). Besides that, Pesavento et al. (2010) also reported that trimethoprim-sulfamethoxazole from antibiotic family group of sulfonamides was highly sensitive (100%) towards *L. monocytogenes* isolates from raw and retail food. Moreover, antibiotics of trimethoprim-sulfamethoxazole was common choice of

replacement if the infected patients are hypersensitive towards the penicillin in the therapy of listeriosis (Alonso-Hernando et al., 2012).

There were 35 antibiotic resistance patterns observed, nearly half (n=61/132, 46.21%) of the tested isolates were multi-drugs resistance and showed MAR index higher than 0.2, as described in **Table 4.5** and **Figure 4.7**. In overall, the MAR indexes that found in the present study were higher than the outcomes (0.38 to 0.63) discovered by Marian et al. (2012) for *L. monocytogenes* isolates from RTE and raw food in Malaysia. Based on **Table 4.5**, there were two isolates out of 132 found to have the highest MAR index value of 0.92 which resistant to 12 antibiotic agents but with slightly different antibiotic resistance patterns. This fact revealed that the evolutionary of *L. monocytogenes* that isolated from food domains are in critical as there might be the occurrence of cross contamination in between food product and environment as they might have the ability of transmission in terms antimicrobial resistance genes (Toomey et al., 2009; Bertsch et al., 2013). Hence, it will be one of the life-threatening issues to the human health as there is an increment in the frequency of antibiotic resistant profiles.

5.4 Biofilm Formation of *L. monocytogenes* in Tryptic Soy Broth (TSB) and Tested Surfaces

Before initiate the biofilm formation study, the growth of *L. monocytogenes* of ATCC 19112 cultured in TSB was observed over time and the OD value is shown in **Figure 4.8**. The OD value grew in the first five hours and peaked at time point 6 h at OD_{600} of 1.055. The value started to decline at time

point 7 h before rising again at 8 h and 9h. Based on the **Figure 4.8**, bacteria's growth was in its $\log_{10}$ phase between 1 h to 6 h.

On the other hand, based on the **Figure 4.9**, *L. monocytogenes* of ATCC 19112 was then grew on stainless-steel coupons of finishing #4 and 2B in order to determine the growth curve of *L. monocytogenes* on the test coupons of size 1.5 × 1.5 × 0.1 cm. Results found that there was an increment in log number of bacteria after 3 hours and reach its highest peak of growth after 6 hours of incubation (#4: 7.91 $\log_{10}$ CFU/cm^2; 2B: 7.68 $\log_{10}$ CFU/cm^2) (**Figure 4.9**). This revealed that initial attachment of biofilm on surface occurred at the first 3 hours and reached its highest peak of growth at 6 hours. At the incubation time of 6 h, the attachment was known as irreversible attachment which initiate the monolayer of biofilm and production of EPS matrix to strengthen the monolayer attachment (Oliveira et al., 2010; Dojiad et al., 2015).

The CFU count decreased between the hours of 6 and 24 and fell to its lowest point during the hours of 24 and 48. As food source was limited for the bacteria for survival, some bacteria cells was degraded when lack of nutrients for multiplication. Then CFU count increased modestly from 48 to 120 hours. At this point, formation of microcolony in multi-layers were taken place (Oliveira et al., 2010; Dojiad et al., 2015). Biofilm formation reached its maturity at 72 h of incubation. Then CFU count decreased from 96 to 120 h which showing the detachment of cells back into planktonic cells (Oliveira et al., 2010; Dojiad et al., 2015).

In order to determine the differences of between the isolates of *L. monocytogenes* of ATCC 19112 and environmental isolate. The isolate in serogroup I.2 which is a strong biofilm former, that consisted seven tested

virulence genes and having MAR index higher than 0.2 which resistant against beta lactams family antibiotics was chosen to conduct in the biofilm studies. In addition, 6 h was chosen as the incubation time for biofilm study as irreversible attachment was formed and considered as pre-mature biofilm.

5.5 Initial Cell Capacity of *L. monocytogenes* in the Biofilm on Test Surfaces

The adherence ability of both isolates was determined based on the initial cell capacity of biofilm on various test surfaces at respective temperature and expressed in $\log_{10}$ CFU/cm^2, as shown in **Table 4.6**. Based on the results, there was a significant difference ($\rho<0.05$) between the cell capacities of ATCC strain and the environmental isolate. The ATCC strain exhibited a slightly higher CFU count than the environmental isolation with estimated marginal means of 8.20±0.21 $\log_{10}$ CFU/cm^2 and 7.81±0.21 $\log_{10}$ CFU/cm^2, respectively. This indicated that ATCC strain adhered better than environmental isolate under the laboratory setting. As ATCC strain was cultured under familiar conditions but environmental isolate was not, thus it may require longer time to reach the similar number of CFU count.

In addition, there is a significant difference ($\rho<0.05$) in CFU count between the stainless-steel and plastic, Teflon and plastic materials but no significant difference ($\rho>0.05$) between the stainless-steel and Teflon material when comparing based on the same strain but in same or different temperature group. This finding revealed that both strains formed better biofilm layer in stainless steel and Teflon surfaces among the three material groups at CFU count approximately of 8 $\log_{10}$ CFU/cm^2. Since both of these materials are high in free

energy surfaces with hydrophilic properties, they are more favourable for bacteria attachment than hydrophobic surface with inert properties such as plastic materials of HDPE and PP (Mafu et al., 1990; Snide and Carballo, 2000). However, biofilm formed better on PP than on HDPE as PP is high porous than HDPE. Bacterial cells able to shelter under pores surfaces (Faille and Carpentier, 2009; Khelissa et al., 2017).

Moreover, there is a significant difference in the same strain of *L. monocytogenes* growth on same or different test surfaces at the incubation temperatures of 4ºC and 25ºC, but not between the 30ºC and 37ºC. For instance, environmental isolate developed slightly more cells on Teflon at temperatures 37ºC (8.33±0.04 $\log_{10}$ CFU/cm^2) and 30ºC (8.26±0.02 $\log_{10}$ CFU/cm^2), than at 4ºC (7.92±0.08 log CFU/cm^2). This indicates that better attachment was formed in 30ºC and 37ºC and showed no significant difference between both temperature as both of these temperatures were the optimum temperature for the growth of *L. monocytogenes* (Colagiorgi et al., 2017).

Therefore, in overall, ATCC strain showed better adherence ability (with CFU count from 8.00 to 8.98 $\log_{10}$ CFU/cm^2) on test surfaces of stainless steel and Teflon material groups incubated at temperature of 30ºC and 37ºC.

5.6 Viable Cell of *L. monocytogenes* on Test Surfaces After Treatment

L. monocytogenes biofilm's viable cell capacity was assessed after the application of disinfection and sanitizing solutions on to the test surfaces in order to have a better understanding about the effectiveness of disinfectant treatment. No significant difference on cell viability between *L. monocytogenes* ATCC 19112 strain and environmental isolate with the observation of ρ-value of 0.32

(>0.05) illustrated in **Table 4.7**. According to the results in **Table 4.7**, the number of viable cells of the ATCC strain and the environmental isolate that remained on Teflon surface that cultured at 4ºC was similarly close which was 5.60 $\log_{10}$ CFU/cm^2 and 5.20 $\log_{10}$ CFU/cm^2, respectively. Results have demonstrated that both strains response in cell reduction towards the disinfection solution.

Additionally, the number of viable cells that persisted on test surfaces varied considerably ($\rho<0.05$) among the three sets of materials, but no significant differences ($\rho>0.05$) observed between the #4 and 2B variations of stainless-steel in both strains. Results had shown less viable cell of environmental strain left on HDPE (4.10 $\log_{10}$ CFU/cm^2) compared to PTFE surface (6.32 $\log_{10}$ CFU/cm^2) even both cultured at 4ºC and treated with SS Sanitizer (SS) solution. Similar observations were in shown in Table 4.7 as less viable cell left on HDPE (6.72 $\log_{10}$ CFU/cm^2) compared to PP (7.03 $\log_{10}$ CFU/cm^2) even both cultured at 37ºC and treated with COS PAA (CP) treatment. Due to the density of HDPE was denser than PP and Teflon, surface low in porosity and hydrophobic to aid the disinfection solution to easily spread through the surfaces to eliminate attached biofilm cell (Faille and Carpentier, 2009; Khelissa et al., 2017).

Nevertheless, there was significant differences in the number of viable cells remained on test surface ($\rho<0.05$) when compared between culturing temperatures. The number of viable ATCC strain cells on #4 stainless-steel surface incubated at 30ºC (7.43 $\log_{10}$ CFU/cm^2) was found to be higher than those incubated at 37ºC (6.88 $\log_{10}$ CFU/cm^2) after the Liquid Sanitizer (LS) treatment. Even though *L. monocytogenes* grew optimally at 30ºC and 37ºC, it can only be highly flagellated and motile at 30ºC than at 37ºC (Peel et al., 1988;

Way et al., 2004). With that, more viable cells were remained on test surfaces that cultured at 30°C even after disinfectant treatment.

Lastly, the lowest viable cell capacity was observed on the treatment with CP solution, followed by LS and SS treatment. Based on the results in **Table 4.7**, the number of viable environmental isolate cells on 2B stainless-steel surface cultivated at 25°C was remained at 6.97 $\log_{10}$ CFU/cm^2, 7.07 $\log_{10}$ CFU/cm^2 and 7.13 $\log_{10}$ CFU/cm^2 after treated with CP, SS and LS solution, respectively. Since CP solution is an acid-based detergent, it comprised active acid compound that can interfere the phospholipid bilayer of bacterial cells and further destroying the cytosolic material of cell via acid oxidation, thus biofilm cell was eliminated from the attached surface (Denyer et al., 1998; Maillard, 2002).

Therefore, it can be concluded that viability of biofilm cell remained on test surface is not varied and showed cell reduction in both strains. Moreover, the least viable cell was observed after CP treatment especially when cultured on dense and hydrophobic surface (HDPE) at lower temperature of 25°C and 4°C.

5.7 Effectiveness of Disinfectant and Sanitizing Solution to treat Biofilm of *L. monocytogenes*

Based on the results in **Table 4.8**, the log reduction between the ATCC strain and environmental isolate differs significantly. The estimated marginal mean value for ATCC strain cell was 1.77±0.22 $\log_{10}$ CFU/cm^2, which is higher than environmental isolate (1.35±0.22 $\log_{10}$ CFU/cm^2). Results indicated there was a larger $\log_{10}$ reduction of cell capacity in ATCC than environmental isolate. Since ATCC strain has not been undergoing any harsh environmental changes along

the growth condition, thus environmental isolate which gained antibacterial effect and antibiotic resistance gene exchanges from its growth origin (e.g.: food matrix) able to show better persistence towards any disinfection treatment (Toomey et al., 2009; Bertsch et al., 2013).

In addition, *L. monocytogenes* cell reduction rates in biofilm of both strains are greatly influenced by cultivation temperature. Log reduction of cell capacity that developed at 4ºC exhibits a larger mean value of reduction when compared to cells grown at 30ºC. The reduction rate of ATCC strain on HDPE surface at 4ºC was found to be 40.05% (2.99 $\log_{10}$ CFU/cm^2), which was significantly higher than ATCC strain on HDPE surface at 30ºC (18.99%, 1.50 $\log_{10}$ CFU/cm^2) after treated with CP solution. Due to higher EPS production in attachment that cultured at higher temperature, Chavant et al. (2002) discovered that *L. monocytogenes* can colonized better on surface material under high growth temperature. Moreover, EPS matrix that produced by biofilm cells at 4ºC is not persist enough to endure the stress from CP treatment. Hence, leading to higher log reduction rate observed in bacterial cells that cultured at high temperature of 30ºC compared to 4ºC.

Besides temperature, test surfaces employed in the study had substantial impact on the log reduction rate of *L. monocytogenes* cell capacity in biofilms. The cell reduction that on surface of 2B stainless steel differs significantly from groups of Teflon and plastic. When compared to the cells number of environment isolate cultured on PTFE (1.12 $\log_{10}$ CFU/cm^2) and HDPE (2.58 $\log_{10}$ CFU/cm^2) at 4ºC after LS treatment, however, the cell reduction on 2B stainless-steel surface at 4ºC was in 0.97 $\log_{10}$ CFU/cm^2, which was noticeably different. Surprisingly, there was no significant difference between the cell reduction

formed on #4 stainless-steel and PP surfaces. For instance, despite the different application of treatment solution, the log reduction of the cell capacity of an environmental isolate produced on #4 stainless steel at 25ºC was 0.99 $\log_{10}$ CFU/cm^2, which is quite similar to the log reduction of the cell grown on PP surface at (0.85 $\log_{10}$ CFU/cm^2). These findings indicate that bacteria cells that grew on stainless steel materials (#4 and 2B) showed the lowest reduction, followed by PP, Teflon and HDPE. Since both stainless steel materials were high in surface energy and hydrophilic on surface, it can hold the attachment of biofilm cells better than Teflon and plastic materials (Mafu et al., 1990; Blackman and Frank, 1996; Hyde et al., 1997).

Lastly, the application of CP solution to the biofilm of *L. monocytogenes* grown at 25ºC demonstrated a highly significant cell reduction rate of 75.17% (6.42 $\log_{10}$ CFU/cm^2) in ATCC strain on PTFE surface when compared to application of LS and SS treatments, which yielded reduction rates of 19.85% (1.70 $\log_{10}$ CFU/cm^2) and 18.19% (1.55 $\log_{10}$ CFU/cm^2), respectively. Result was in line with the previous study, 5 log reduction was determined when biofilm treated with 0.1% combination of peracetic acid and hydrogen peroxide with the exposure of 10 min (Brinez et al., 2006). Hence, CP treatment was determined to be the most effective disinfectant and sanitizing method in current investigation, followed by SS and LS treatments to eliminate the growth of *L. monocytogenes* biofilm that were grown on stainless-steel, Teflon and plastic material in cultivation temperature of 4ºC, 25ºC, 30ºC and 37ºC.

5.8 Scanning Electron Microscopy (SEM)

Based on the results (**Figure 4.10** and **Figure 4.13**), both strains able attached to #4 stainless steel surfaces and formed honey-comb structures uniformly. However, higher density of honey-comb structures was observed on all the test coupons of (A) to (D) by ATCC strain when compared to (M) to (P) by environmental strain. This observation is a visual supportive fact to explain that ATCC strain grew better on stainless steel surface than environmental isolate as the growing condition provided was a in laboratory condition that not familiar to environmental isolates. Hence, higher growing rate was shown in **Figure 4.10** than in **Figure 4.13**. Besides that, test coupons (A), (B), (M) and (N) showed complex honey-comb structure than test coupons (C), (D), (O) and (P). This can be explained that both incubation temperature is the optimum temperature (37ºC and 30ºC) that supports the growth of *L. monocytogenes* when compared to 25ºC and 4ºC (Colagiorgi et al., 2017).

The overview of the initial attachment of both ATCC strain and environmental isolate that viewed under a higher magnification as shown in **Figure 4.11** and **Figure 4.14**, respectively. By comparing the EPS production from both strains, ATCC showed higher EPS production than environmental isolate. Formation of microcolonies were observed in **Figure 4.11** and **Figure 4.14**, indicated the formation of monolayer of biofilm layer in attachment (Muhammad et al., 2020). Under incubation time of 4ºC, both isolates formed abnormal cells (some short and small and some was elongated) that demonstrated that cells were undergoing thermal stress under low growing temperature.

Lastly, based on the **Figure 4.12** and **Figure 4.13**, these illustrated the effectiveness of CP treatment in removing biofilm cells by damaging the attached biofilm cells into crevices in shape or break into pieces (Denyer et al., 1998; Maillard, 2002). There were some injury cells were shown in test coupon (W), indicating some cells able to endure the stress from CP treatment not being destroyed. Yet there were a lot of damaged cells in test coupons (J) and (K) where the phospholipid bilayer of bacterial cells destroyed and left shrinkage cells on the monolayer formed. The observation in Figure 4.12 showed the visualized cell damage conditions which being discussed in section 5.7, whereby higher efficacy shown in eliminating cells grew by ATCC strain than environmental isolate after treated with CP treatment.

5.9 Future Study

Based on the findings that discussed above and the outbreaks that reported in most of the developed countries in recent years, there are still numerous unknowns' information about *L. monocytogenes* to be discovered in the future. One of the interesting topics to be studied in the future perspectives is the determination of whole genome sequences of novel isolates from serogroup III. Pathogenicity of *L. monocytogenes* not only just focus on those strains that have been known and commonly isolated from food, environmental and human clinical sources. It is worth to discuss these interesting fact as there is an increment in the frequency of antibiotic resistance isolates that with the attention of the global disease outbreak.

Besides that, the biofilm forming ability of mixed culture with *L. monocytogenes* from different serogroups or mixed culture with other novelty

foodborne pathogen such as *Salmonella* Typhimurium, *Campylobacter* and others also recommended to be carried out in the future. It can be considered as the actual scenario that may present in the food domains, whereby bacterial cells may require to compete among others for survival especially under the extreme conditions in food processing environment, for instance, daily cleaning section.

Subsequently, another important aspect in future study was the techniques invented for biofilm removal that must be environmentally friendly, for instance, implementation of enzymatic disinfection method. Implementation of enzymatic disinfection technique to mixed culture biofilms not only can remove the biofilm via biological method but it also able to overcome the issue of over-usage of antibiotics and chemical agents that may aid the evolutionary of *L. monocytogenes* to become more virulent and causes unwanted severe listeriosis outbreaks to happen.

CHAPTER 6

CONCLUSION

In conclusion, there are more *Listeria* spp. and *L. monocytogenes* isolates were isolated from food samples than food contact surfaces, whereby higher prevalence count was found in processed food among the four food categories and direct food contact surfaces from both food contact surface categories.

According to the present study, most collected isolates were belonging to serogroup II.1 which consisting of virulence genes of *inl*A, *inl*C, *inl*J, *plc*A, a*ct*A, *hly*A and *iap* genes that associated with listeriosis and multidrug-resistant towards the common antibiotics that are used to treat listeriosis.

For the biofilm forming adherence ability, *L. monocytogenes* strain of ATCC 19112 showed higher adherence ability on stainless steel (#4 and 2B) and Teflon surface materials when incubated under laboratory setting at 30ºC and 37ºC than the environmental isolate. However, environment isolate that grew on stainless steel surfaces of stainless steel with grading of #4 and 2B which incubated at 30ºC were found to be more persistent and exhibited less efficiency in acid treatment when compared to the strain of ATCC 19112.

Therefore, it is important to introduce a proper and traceable health surveillance system to the public and educate food handlers about the proper cleaning practices in order to lower down the risk of foodborne pathogen growth

at food domains in Malaysia and subsequently cross-contaminate the end-product.

REFERENCES

Adnan, K., Chughtai, M.F.J., Mehmood, T., Ahsan, S., Liaqat, A., Nadeem, M., Sameed, N., Saeed, K., Rehman, J.U. and Ali, A., 2021. High Pressure Processing; Principle, Applications, Impact and Future Prospective. *Sustainable Food Processing and Engineering Challenges*, Chapter 3, pp. 75-108. doi: 10.1016/B978-0-12-822714-5.00003-6

Adrião, A., Vieria, M. Fernandes, I, Barbosa, M. Sol, M., Tenreiro, R.P., Chambel, L., Barata, B., Zilhao, I, Shama, G., Perni, S., Jordan, S.J., Andrew, P.W. and Faleiro, M.L., 2008. Marked intra-strain variation in response of *Listeria monocytogenes* dairy isolates to acid or salt stress and the effect of acid or salt adaptation on adherence to abiotic surfaces. *International Journal of Food Microbiology*, 123(1-2), pp. 142-150. doi: 10.1016/j.ijfoodmicro.2007.12.016

Akabanda, F., Hlortsi, E.H., Owusu-Kwarteng, J., 2017. Food safety knowledge, attitudes and practices of institutional food-handlers in Ghana. *BMC Public Health*, 17, pp. 40. doi: 10.1186/s12889-016-3986-9

Allerberger, F., 2003. *Listeria*: growth, phenotypic differentiation and molecular microbiology. *FEMS Immunology and Medical Microbiology*, 35(3), pp. 183-189. doi: 10.1016/S0928-8244(02)00447-9

Al-Nabulsi, A., Osaili, T.M., Awad, A.A., Olaimat, A.N., Shaker, R.R. and Holley, R.A., 2015a. Occurrence and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw and processed meat products in Amman, Jordania. *CyTA - Journal of Food*, 13(3), pp. 346-352. doi: 10.1080/19476337.2014.982191

Al-Nabulsi, A.A., Osaili, T.M., Shaker, R.R., Olaimat, A.N., Jaradat, Z.W., Elabedeen, N.A. and Holley, R.A., 2015b. Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of *Listeria monocytogenes*. *Food Microbiology*, 46, pp. 154-160. doi: 10.1016/j.fm.2014.07.015

Alonso-Hernando, A., Prieto, M, García-Fernández, C., Alonso-Calleja, C. and Capita, R., 2012. Increase over time in the prevalence of multiple antibiotic resistance among isolates of *Listeria monocytogenes* from poultry in Spain. *Food Control*, 23(1), pp. 37-41. doi: 10.1016/j.foodcont.2011.06.006

Alvarez-Ordóñez, A., Leong, D. Morgan, C.A., Hill, C., Gahan, C.G.M. and Jordan, K., 2015. Occurrence, Persistence, and Virulence Potential of *Listeria ivanovii* in Foods and Food Processing Environments in the Republic of Ireland. *BioMed Research International*, 2015, pp. 1-10. doi: 10.1155/2015/350526

Amusan, E.E. and Sanni, A.I., 2019. Isolation and Identification of *Listeria monocytogenes* in Fresh Croaker (*Pseudotolithus senegalensis*). *IOP Conference Series: Earth and Environmental Science*, 210, pp. 012004. doi: 10.1088/1755-1315/210/1/012004

Andriyanov, P.A., Zhurilov, P.A., Liskova, E.A., Karpova, T.I., Sokolova, E.V., Yushina, Y.K., Zaiko, E.V., Baraeva, D.S., Voronina, O.L., Psareva, E.K., Tartakovsky, I.S., Kolbasov, D.V. and Ermolaeva, S.A., 2021. Antimicrobial Resistance of Listeria monocytogenes Strains Isolated from Humans, Animals and Food Products in Russia in 1950-1980, 2000-2005, and 2018-2021. *Antibiotics*, 10(10), pp. 1206. doi: 10.3390/antibiotics10101206

Arumugaswamy, R.K., Rahmat Ali, G.R. and Hamid, S.N., 1994. Prevalence of *Listeria monocytogenes* in foods in Malaysia. *International Journal of Food Microbiology*, 23(1), pp. 117-121. doi: 10.1016/0168-1605(94)90227-5

Augustin, M., Ali-Vehmas, T. and Atroshi, F., 2004. Assessment of enzymatic cleaning agents and disinfectants against bacterial biofilms. *Journal of Pharmacy and Pharmaceutical Sciences*, 7(1), pp. 55-64. PMID: 15144735

Aureli, P., Fiorucci, G.C., Caroli, D., Marchiaro, G., Novara, O., Leone, L. and Salmosa, S., 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. *The New England Journal of Medicine*, 342(17), pp. 1236-1241. doi: 10.1056/NEJM200004273421702

Azizoglu, R.O., Osborne, J., Wilson, S. and Kathariou, S., 2009. Role of growth temperature in freeze-thaw tolerance of Listeria spp. *Applied and Environmental Microbiology*, 75(16), pp. 5315-5320. doi: 10.1128/AEM.00458-09

Bae, D., Liu, C., Zhang, T., Jones, M., Peterson, S.N. and Wang, C., 2012. Global gene expression of *Listeria monocytogenes* to salt stress. *Journal of Food Protection*, 75(5), pp. 906-912. doi: 10.4315/0362-028X.JFP-11-282

Bansal, V., Siddiqui, M.W. and Rahman, M.S., 2015. Minimally Processed Foods: Overview. In Siddiqui, M. and Rahman, M. (Eds). Minimally Processed Foods. *Food Engineering Series*, p.1-15. Switzerland AG: Springer. doi: 10.1007/978-3-319-10677-9_1

Barbosa, J., Borges, S., Camilo, R., Magalhães, R., Ferreira, V., Santos, I., Silva, J., Almeida, G. and Teixeira, P. (2013). Biofilm Formation among Clinical and Food Isolates of *Listeria monocytogenes*. *International Journal of Microbiology*, 2013, pp. 524975. doi: 10.1155/2013/524975

Barria, C., Malecki, M. and Arraiano, C.M., 2013. Bacterial adaption to cold. *Microbiology Society*, 159(2), pp. 2437-2443. doi: 10.1099/mic.0.052209-0

Bayles, D.O. and Wilkinson, B.J., 2000. Osmoprotectants and cryoprotectants for *Listeria monocytogenes*. *Letters in Applied Microbiology*, 30(1), pp. 23-27. doi: 10.1046/j.1472-765x.2000.00646.x

Beales, N., 2004. Adaption of Microorganisms to Cold Temperatures, Weak Acid Preservatives, Low pH, and Osmotic Stress: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 3(1), pp. 1-20. doi: 10.1111/j.1541-4337.2004.tb00057.x.

Begley, M., Kerr, C. and Hill, C., 2009. Exposure to bile influences biofilm formation by *Listeria monocytogenes*. *Gut Pathogens*, 1, pp. 1-4. doi: 10.1186/1757-4749-1-11

Berrang, M.E., Frank, J.F. and Meinersmann, R.J., 2013. Contamination of raw poultry meat by airborne *Listeria* originating from a floor drain. *The Journal of Applied Poultry Research*, 22(1), pp. 132-136. doi: 10.3382/japr.2012-00676

Bergkessel, M. and Guthrie, C., 2013. Colony PCR. *Methods in Enzymology: DNA*, 529, pp. 299-309. doi: 10.1016/B978-0-12-418687-3.00025-2

Bertsch, D., Uruty, U.M., Anderegg, J., Lacroix, C. and Meile, L., 2013. Tn6198, a noval transposon containing the trimethoprim resistance gene dfrG embedded into a Tn916 element in *Listeria monocytogenes*. *Journal of Antimicrobial Chemotherapy*, 68(5), pp. 986-991. Doi: 10.1093/jac/dks531

Beye, M., Gouriet, F., Michelle, C., Casalta, J.P., Habib, G., Raoult, D. and Fournier, P.E., 2016. Genome analysis of *Listeria ivanovii* strain G770 that caused a deadly aortic prosthesis infection. *New Microbes and New Infections*, 10, pp. 87-92. doi: 10.1016/j.nmni.2016.01.005

Blackman, I.C. and Frank, J.F., 1996. Growth of *Listeria monocytogenes* as a biofilm on various food-processing surfaces. *Journal of Food Protection*, 59(8), pp. 827-831. doi: 10.4315/0362-028X-59.8.827

Bohdan, A., 2023. *Listeria monocytogenes in Fresh And Ready-to-eat Produce: Common Sources and Potential Hazards*, [online] Available at: < https://blog.nemistech.com/en/listeria-monocytogenes-in-fresh-and-ready-to-eat-produce-common-sources-and-potential-hazards> [Accessed 29 December 2023].

Bojsen-Møller, J., 1972. Human listeriosis. Diagnostic, epidemiological and clinical studies. *Acta pathologica et microbiologica Scandinavica, Section B: Microbiology and immunology*, 229, pp. 12-43. PMID: 4624477

Bonaventure, G.B., Piccolomini, R., Paludi, D., D'Orio, V., Vergara, A., Conter, M. and Ianieri, A., 2008. Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: relationship with motility and cell surface hydrophobicity. *Journal of Applied Microbiology*, 104(6), pp. 1552-1561. doi: 10.1111/j.1365-2672.2007.03688.x

Bonsagila, E.C.R., Silva, N.C.C., Fernades Junior, A., Araújo Júnior, J.P., Tsunemi, M.H. and Rall, V.L.M., 2014. Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. *Food Control*, 35(1), pp. 386-391. doi: 10.1016/j.foodcont.2013.07.023

Borucki, M.K., Peppin, J.D., White, D., Loge, F. and Call, D.R., 2003. Variation in biofilm formation among strains of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 69(12), pp. 7336-7342. doi: 10.1128/AEM.69.12.7336-7342.2003

Bower, C.K., McGuire, J. and Daeschel, M.A., 1995. Suppression of *Listeria monocytogenes* colonization following adsorption of nisin onto silica surfaces. *Applied and Environmental Microbiology*, 61, pp. 992-997. doi: 10.1128/aem.61.3.992-997.1995

Borucki, M.K., Kim, S.H., Call, D.R., Smole, S.C. and Pagotto, F., 2004. Selective Discrimination of *Listeria monocytogenes* Epidemic Strains by a Mixed-Genome DNA Microarray compared to Discrimination by Pulse-Field Gel Electrophoresis, Ribotyping, and Multilocus Sequence Typing. *Journal of Clinical Microbiology*, 42(11), pp. 5270-5276. doi: 10.1128/JCM.42.11.5270-5276.2004

Boscher, E., Houard, E. and Denis, M., 2012. Prevalence and distribution of *Listeria monocytogenes* serotypes and pulsotypes in sows and fattening pigs in farrow-to-finish farms (France, 2008). *Journal of Food Protection*, 75(5), pp. 889-895. doi: 10.4315/0362-028X.JFP-11-340

Braga, V., Vázquez, S., Vico, V., Pastorino, V., Mota, M.I., Legnani, M., Schelotto, F., Lancibidad, G. and Varela, G., 2017. Prevalence and serotype distribution of *Listeria monocytogenes* isolated from foods in Montevideo Uruguay. *Food Microbiology*, 48(4), pp. 689-694. doi: 10.1016/j.bjm.2017.01.010

Bremer, P.J., Monk, I. and Butler, R., 2002. Inactivation of *Listeria monocytogenes*/*Flavobacterium* spp. biofilms using chlorine: Impact of substrate, pH, time and concentration. *Letter of Applied Microbiology*, 35(4), pp. 321-325. doi: 10.1046/j.1472-765x.2002.01198.x

Brinez, W.J., Roig-Sagues, A.X., Manuela, H.H.M., Lopez-Pedemonte, T. and Guamis, B., 2006. Bactericidal efficacy of peracetic acid in combination with hydrogen peroxide against pathogenic and non-pathogenic strains of *Staphylococcus* spp., *Listeria* spp. and *Escherichia coli*. *Food Control*, 17, pp. 516–521. doi: 10.1016/j.foodcont.2005.02.014

Bruhn, J.B., Vogel, B.F. and Gram, L., 2005. Bias in the *Listeria monocytogenes* Enrichment Procedure: Lineage 2 Strains Outcompete Lineage 1 Strains in University of Vermont Selective Enrichments. *Applied and Environmental Microbiology*, 71(2), pp. 961-967. doi: 10.1128/AEM.71.2.961-967.2005

Burn, C.G., 1935. Characteristics of a new species of the genus Listerella obtained from human sources. *Journal of Bacteriology*, 30(6), pp. 573-591. doi: 10.1128/jb.30.6.573-591.1935

Butts, J., 2003. *Seek & destroy: identifying and controlling Listeria monocytogenes growth niches*, [online] Available at: <https://www.food-safety.com/articles/4802-seek-destroy-identifying-and-controlling-listeria-monocytogenes-growth-niches> [Accessed 8 February 2023].

Camargo, A.C., Woodward, J. J., Call, D.R. and Nero, L.A., 2017. *Listeria monocytogenes* in Food Processing Facilities, Food Contamination, and Human Listeriosis: The Brazilian Scenario. *Foodborne Pathogens and Disease*, 14(11), pp. 623-636. doi: 10.1089/fpd.2016.2274

Carpentier, B. and Cerf, O., 2011. Review-Persistence of Listeria monocytogenes in food industry equipment and premises. International Journal of Food Microbiology, 145(1), pp. 1-8. doi: 10.1016/j.ijfoodmicro.2011.01.005

Caroline, 2023. *Cooking Vegetables at the Perfect Temperature and Time – A Guide*, [online] Available at: <https://temperatureask.com/what-temperature-should-vegetables-be-cooked-at/> [Accessed 3 February 2023].

Castro, H.K. and Lindström, M., 2016. Ecology and surveillance of *Listeria monocytogenes* on dairy cattle farms. *International Journal of Infectious Diseases*, 53, pp. 68. doi: 10.1016/j.ijid.2016.11.171

Cataldo, G., Conte, M.P., Chiarini, F., Seganti, L., Ammendolia, M.G., Superti F. and Longhi, C., 2007. Acid adaptation and survival of *Listeria monocytogenes* in Italian-style soft cheese. *Journal of Applied Microbiology*, 103, pp. 185-193. doi: 10.1111/j.1365-2672.2006.03218.x

Chaitiemwong, N., Hazeleger, W.C., Beumer, R.R. and Zwietering, M.H., 2014. Quantification of transfer of *Listeria monocytogenes* between cooked ham and slicing machine surfaces. *Food Control*, 44, pp. 177-184. doi: 10.1016/j.foodcont.2014.03.056

Chavant, P., Martinie, B., Meylheuc, T., Bellon-Fontaine, M. and Hebraud, M., 2002. *Listeria monocytogenes* LO28: surface physicochemical properties and ability to form biofilms at different temperature and growth phases. *Applied and Environmental Microbiology*, 68(2), pp. 728-737. doi: 10.1128/AEM.68.2.728-737.2002

Chen, C.Y., Nace, G.W. and Irwin, P.L., 2003. A 6×6 drop plate method for simultaneous colony counting and MPN enumeration of *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli*. *Journal of Microbiological Methods*, 55(2), pp. 475-479. doi: 10.1016/s0167-7012(03)00194-5

Chen, J., Zhang, X., Mei, L., Jiang, L. and Fang, W., 2009a. Prevalence of *Listeria* in Chinese Food Products from 13 Provinces Between 2000 and 2007 and Virulence Characterization of *Listeria monocytogenes* Isolates. *Foodborne Pathogens and Disease*, 6(1), pp. 7-14. doi: 10.1089/fpd.2008.0139

Chen, J., Jiang, L., Chen, X., Luo, X., Chen, Y., Yu, Y., Tian, G., Liu, D. and Fang, W., 2009b. *Listeria monocytogenes* Serovar 4a is a Possible Evolutionary Intermediate Between *L. monocytogenes* Serovars 1/2a and 4b and *L. innocua*.

Journal of Microbiology and Biotechnology, 19(3), pp. 238-249. doi: 10.4014/jmb.0805.304

Chen, J., Cheng, C., Xia, Y., Zhao, H., Fang, C., Shan, Y., Wu, B. and Fang, W., 2011. Lmo0036, an ornithine and putrescine carbamoyltransferase in *Listeria monocytogenes*, participates in arginine deiminase and agmatine deiminase pathways and mediates acid tolerance. *Microbiology Society*, 157(11), pp. 3150-3161. doi: 10.1099/mic.0.049619-0

Chen, Y., Pouillot, R., Burall, L., Strain, E.A., Van Doren, J.M., De Jesus, A.J., Laasri, A., Wang, H., Ali, L., Tatavarthy, A. Zhang, G., Hu, L., Day, J., Sheth, I., Kang, J., Sahu, S., Srinivasan, D., Brown. E.W., Parish, M., Zink, D., Datta, A.R., Hammack, T.S. and Macarisin, D., 2016. Comparative evaluation of direct plating and most probable number methods for the enumeration of low levels of *Listeria monocytogenes* in naturally contaminated ice cream products. *International Journal of Food Microbiology*, 241, pp. 15-22. doi: 10.1016/j.ijfoodmicro.2016.09.021

Chen, J.Q., Regan, P., Laksanalamai, P., Healey, S. and Hu. Z., 2017. Prevalence and methodologies for detection, characterization and subtyping of *Listeria monocytogenes* and *L. ivanovii* in foods and environmental sources. *Food Science and Human Wellness*, 6(3), pp. 97-120. doi: 10.1016/j.fshw.2017.06.002

Cheng, Y., Dong, Q., Liu, Y., Liu, H., Zhang, H. and Wang, X., 2022. Systematic review of *Listeria monocytogenes* from food and clinical samples in Chinese mainland from 2010 to 2019. *Food Quality and Safety*, 6. doi: 10.1093/fqsafe/fyac021

Chevallier, I., Ammor, S., Laguet, A., Labayle, S., Castanet, V., Dufour, E. and Talon, R., 2006. Microbial ecology of a small-scale facility producing traditional dry sausage. *Food Control*, 17(6), pp. 446-453. doi: 10.1016/j.foodcont.2005.02.005

Chin, P.S., Ang, G.Y., Yu, C.Y., Tan, E.L., Tee, K.K., Yin, W.F., Chan, K.G. and Tan, G.Y.A., 2018. Prevalence Antimicrobial Resistance, and Genetic Diversity of *Listeria* spp. Isolated from Raw Chicken Meat and Chicken Related Products in Malaysia. *Journal of Food Protection*, 81(2), pp. 284-289. doi: 10.4315/0362-028X.JFP-17-186

Chmielewski, R.A.N. and Frank, J.F., 2003. Biofilm Formation and Control in Food Processing Facilities. *Comprehensive Reviews in Food Science and Food Safety*, 2(1), pp. 22-32. doi: 10.1111/j.1541-4337.2003.tb00012.x

Chorianopoulos, N. Giaouris, E., Grigoraki, I., Skandamis, P. and Nychas, G.J., 2011. Effect of acid tolerance response (ATR) on attachment of *Listeria monocytogenes* Scott A to stainless steel under extended exposure to acid or/and salt stress resistance of sessile cells to subsequent strong acid challenge. *International Journal of Food Microbiology*, 145(2-3), pp. 400-406. doi: 10.1016/j.ijfoodmicro.2011.01.001

Christen, J.A. and Parker, A.E., 2020. Systematic statistical analysis of microbial data from dilution series. *Journal of Agricultural, Biological and Environmental Statistics*, 25, pp. 339-364. doi: 10.1007/s13253-020-00397-0

Center for Food Safety and Applied Nutrition (CFSAN), 2017. *Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration.* [online] Available at: <https://www.fda.gov/files/food/published/Draft-Guidance-for-Industry--Control-of-Listeria-monocytogenes-in-Ready-To-Eat-Foods-%28PDF%29.pdf> [Accessed 8 February 2023]

Clinical Laboratory Standards Institute (CLSI)., 2020. *Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100*. Wayne, PA. Clinical and Laboratory Standards Institute.

Colagiorgi, A., Bruini, I., Ciccio, P.A.D., Zanardi, E., Ghidini, S. and Ianieri, A., 2017. *Listeria monocytogenes* Biofilms in the Wonderland of Food Industry. *Pathogens*, 6 (3), pp. 41-50. doi: 10.3390/pathogens6030041

Combrouse, T., Sadovskaya, I., Faille, C., Kol, O., Guérardel, Y. and Midelet-Bourdin, G., 2013. Quantification of the extracellular matrix of the *Listeria monocytogenes* biofilms of different phylogenic lineages with optimization of culture conditions. *Journal of Applied Microbiology*, 114(4), pp. 1120-1131. doi: 10.1111/jam.12127

Cordero, N., Maza, F., Navea-Perez, H., Aravena, A., Marquez-Fontt, B., Navarrete, P., Figueroa, G., González, M., Latorre, M. and Reyes-Jara, A., 2016. Different Transcriptional Responses from Slow and Fast Growth Rate Strains of *Listeria monocytogenes* Adapted to Low Temperature. *Frontiers in Microbiology*, 7, pp. 229. doi: 10.3389/fmicb.2016.00229

Cotter, P.D., Gahan, G.C. and Hill, C., 2000. Analysis of the role of the *Listeria monocytogenes* F_0F_1-AtPase operon in the acid tolerance response. International *Journal of Food Microbiology*, 60(2-3), pp. 137-146. doi: 10.1016/s0168-1605(00)00305-6

Cotter, P.D., Gahan, C.G. and Hill, C., 2001. A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. *Molecular Microbiology*, 40(2), pp. 465-475. doi: 10.1046/j.1365-2958.2001.02398.x.

Cotter, P.D. and Hill, C., 2003. Surviving the Acid Test: Responses of Gram-Positive Bacteria to Low pH. *Microbiology and Molecular Biology Reviews*, 67(3), pp. 429-453. doi: 10.1128/MMBR.67.3.429-453.2003

Curtin, J.J. and Donlan, R.M., 2006. Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis*. *Antimicrobial Agents and Chemotherapy*, 50(4), pp. 1268-1275. doi: 10.1128/AAC.50.4.1268-1275.2006

Cutter, C.N., 2017. *Control of Listeria monocytogenes in Retail Establishments. College of Agricultural Sciences, Agricultural Research and Cooperative Extension, Pennsilvania State University.* [online] Available at: <https://extension.psu.edu/control-of-listeria-monocytogenes-in-retail-establishments> [Accessed 8 February 2023].

Dabney, J., Meyer, M. and Pääbo, S., 2013. Ancient DNA Damage. *Cold Spring Harbor Perspectives in Biology*, 5(7), a012567. doi: 10.1101/cshperspect.a012567

Davis, M.J., Coote, P.J. and O'Byrne, C.P., 1996. Acid tolerance in *Listeria monocytogenes*: the adaptive acid tolerance response (ATR) and growth-phase-dependent acid resistance. *Microbiology Society*, 142(10), pp. 2975-2982. doi: 10.1099/13500872-142-10-2975

De Cesare, A., Mafreda, G., Macri, M. and Cantoni, C., 2007. Application of automated ribotyping to support the evaluation of *Listeria monocytogenes* sources in a Taleggio cheese producing plant. *Journal of Food Protection*, 70(5), pp. 1116-1121. doi: 10.4315/0362028X-70.5.1116

Denyer, S.P. and Stewart, G.S.A.B., 1998. Mechanisms of action of disinfectants. *International Biodeterioration and Biodegradation*, 41(3-4), pp. 261-268. doi: 10.1016/S0964-8305(98)00023-7

Djordjevic, D.E., Wiedmann, M. and McLandsborough, L.A., 2002. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Applied*

and Environmental Microbiology, 68(6), pp. 2950-2958. doi: 10.1128/AEM.68.6.2950-2958.2002

Dojiad, S.P., Poharkar, K.V., Kale, S.B., Kerkar, S., Kalorey, D.R., Kurkure, N.V., Rawool, D.B., Malik, S.V.S., Ahmad, R.Y., Hudel, M., Chaudhari, S.P., Abt, B., Overmann, J., Weigel, M., Hain, T., Barbuddhe, S.B. and Chakraborty, T., 2018. Taxonomic description *Listeria goaensis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 68(10), pp. 3285-3291. doi: 10.1099/ijsem.0.002980

Donlan, R.M. and Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical Microbiology Reviews*, 15 (2), pp. 167–193. doi: 10.1128/CMR.15.2.167-193.2002

Dowe, M.J., Jackson, E.D., Mori, J.G. and Bell, C.R., 1997. *Listeria monocytogenes* Survival in Soil and Incidence in Agricultural Soils. *Journal of Food Protection*, 60(10), pp. 1201-1207. doi: 10.4315/0362-028X-60.10.1201

Doumith, M., Cazalet, C., Simoes, N., Frangeul, L. and Jacquet, C., 2004a. New aspects regarding evolution and virulence of *Listeria monocytogenes* revealed by comparative genomics and DNA arrays. *Infection and Immunity*, 72, 1072-1083. doi: 10.1128/IAI.72.2.1072-1083.2004

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P., 2004b. Differentiation of the Major *Listeria monocytogenes* Serovars by Multiplex PCR. *American Society for Microbiology Journal of Clinical Microbiology*, 42(8), pp. 3819-3822. doi: 10.1128/JCM.42.8.3819-3822.2004

Doyle, M.E. and Glass, K.A., 2010. Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety*, 9(1), pp. 44-56. doi: 10.1111/j.1541-4337.2009.00096.x

Du, X., Zhang, X., Wang, X., Su, Y., Li, P. and Wang, S., 2017. Isolation and characterization of *Listeria monocytogenes* in Chinese food obtained from the central area of China. *Food Control*, 74, pp. 9-16. doi: 10.1016/j.foodcont.2016.11.024

Dumen E., Baca, A.U. and Dumen, E., 2008. Comparative detection of *Listeria monocytogenes* in raw milk by microbiological method and PCR. *Medycyna Weterynaryjna*, 64(1), pp. 59–63.

Dutta, V., Elhanaf, D. and Kathariou, S., 2013. Conservation and distribution of the benzalkonium chloride resistance cassette bcrABC in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 79(19), pp. 6067-6074. doi: 10.1128/AEM.01751-13

Ennaji, H., Timinouni, M., Ennaji, M.M., Hassar, M. and Cohen, N., 2008. Characterization and antibiotic susceptibility of *Listeria monocytogenes* isolated from poultry and red meat in Morocco. *Infection and Drug Resistance*, 1, pp. 45-50. doi: 10.2147/idr.s3632

Endrikat, S., Gallagher, D., Pouillot, R., Quesenberry, H.H., Labarre, D., Schroeder, C.M. and Kause, J., 2010. A comparative risk assessment for *Listeria monocytogenes* in prepackaged versus retail-sliced deli meat. *Journal of Food Protection*. 73(4), pp. 612-619. doi: 10.4315/0362-028x-73.4.612

Ermolaeva, S., Karpova, T., Novella, S., Wagner, M., Scortti, M., Tartakovskii, I. and Vazquez-Boland, J.A., 2003. A simple method for the differentiation of *Listeria monocytogenes* based on induction of lecithinase activity by charcoal. *International Journal of Food Microbiology*, 82(1), pp. 87-94. doi: 10.1016/s0168-1605(02)00399-9

European Food Safety Authority (EFSA). , 2013. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates. *EFSA Journal*, 11(6), pp. 3241. doi: 10.2903/j.efsa.2013.3241

Escolar, C., Gómez, D., García, M.D.C.R., Conchello, P. and Herrera, A., 2017. Antimicrobial resistance profiles of *Listeria monocytogenes* and *Listeria innocua* isolated from ready-to-eat products of animal origin in Spain. *Foodborne Pathogens and Disease*, 14(6), pp. 357-363. doi: 10.1089/fpd.2016.2248

Faille, C. and Carpentier, B., 2009. *Food contact surfaces, surface soiling and biofilm formation*, pp. 301-330. In Fratamico P.M., Annous, B.A. and Gunther, N.W., (IV) ed., Biofilms in the food and beverages industries. Woodhead Publishing, Cambridge, United Kingdom.

Farber, J.M. and Peterkin, P.I., 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiology Reviews*, 55(3), pp. 476-511. doi: 10.1128/MR.55.3.476-511.1991

Food and Drug Administration., 2012. *Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook – Listeria monocytogenes*. [online] Available at: <https://www.fda.gov/files/food/published/Bad-Bug-Book-2nd-Edition-%28PDF%29.pdf> [Accessed 8 February 2023].

Food and Drug Administration., 2019. *Listeria (Listeriosis)*, [online] Available at: <https://www.fda.gov/food/foodborne-pathogens/listeria-listeriosis> [Accessed 5 December 2021].

Franciosa, G., Tartaro, S., Wedell-Neergaard, C. and Aureli, P., 2001. Characterization of *Listeria monocytogenes* strains involved in invasive and noninvasive listeriosis outbreaks by PCR-based fingerprinting techniques. *Applied and Environmental Microbiology*, 67(4), pp. 1793-1799. doi: 10.1128/AEM.67.4.1793-1799.2001

Fredriksson-Ahomaa, M., Gerhardt, M. and Stolle, A., 2009. High bacterial contamination of pig tonsils at slaughter. *Meat Science*, 83 (2), pp. 334-336. doi: 10.1016/j.meatsci.2009.06.004

Fredslund, L., Ekelund, F., Jacobsen, C.S. and Johnsen, K., 2001. Development and application of a most-probable-number-PCR assay to quantify flagellate populations in soil samples. *Applied and Environmental Microbiology*, 67(4), pp. 1613-1618. doi: 10.1128/AEM.67.4.1613-1618.2001

Freezing Technology, 2011. *Microbial growth at low temperature*. [online] Avilable at: <http://ecoursesonline.iasri.res.in/mod/page/view.php?id=146131> [Accessed 8 February 2023].

Folsom, J.P., Siragusa, G.R. and Frank, J.F., 2006. Formation of biofilm at different nutrient by various genotypes of *Listeria monocytogenes*. *Journal of Food Protection*, 69(4), pp. 826-834. doi: 10.4315/0362-028x-69.4.826

Fontana, C., Cocconcelli, P.S., Vignolo, G. and Saavedra, L., 2015. Occurrence of antilisterial structural bacteriocins genes in meat borne lactic acid bacteria. *Food Control*, 47, pp. 53-59. doi: 10.1016/j.foodcont.2014.06.021

Fox, E.M., Soloman, K., Moore, J.E., Wall, P.G. and Fanning, S., 2014. Phylogenetic profiles of in-house microflora in drains at a food production facility: Comparison and biocontrol implications of listeria-positive and negative bacterial populations. *Applied and Environmental Microbiology*, 80, pp. 3369-3374. doi: 10.1128/AEM.00468-14

Garrec, N., Picard-Bonnaud, F. and Pourcher, A.M., 2003. Occurrence of *Listeria* sp. and *L. monocytogenes* in sewage sludge used for land application: effect of dewatering, liming and storage in tank on survival of *Listeria* species. *FEMS Immunology and Medical Microbiology*, 35(3), pp. 275-283. doi: 10.1016/S0928-8244(02)00443-1

Gasanov, U., Hughes, D. and Hansbro, P.M., 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. *FEMS Microbiology Reviews*, 29(5), pp. 851-875. doi: 10.1016/j.femsre.2004.12.002

Gerba, C.P., 2015. Quaternary Ammonium Biocides: Efficacy in Application. *Applied and Environmental Microbiology*, 81, pp. 464-469. doi: 10.1128/AEM.02633-14

Giaouris, E., Chapot-Chartier, M.P. and Briandet, R., 2009. Surface physicochemical analysis of natural Lactococcus lactis strains reveals the existence of hydrophobic and low charged strains with altered adhesive properties. *International Journal of Food Microbiology*, 131, pp. 2-9. doi: 10.1016/j.ijfoodmicro.2008.09.006

Goh, S.G., Kuan, C.H., Loo, Y.Y., Chang, W.S., Lye, Y.L., Soopna, P., Tang, J.H.Y., Nakaguchi, Y., Nishibuchi, M., Afsah-Hejri, L. and Son, R., 2012. *Listeria monocytogenes* in retailed raw chicken meat in Malaysia. *Poultry Science*, 91(10), pp. 2686-2690. doi: 10.3382/ps.2012-02349

Goulet, V., Hebert, M., Hedberg, C., Laurent, E., Vaillant, V., De Valk, H. and Desenclos, J.C., 2012. Incidence of Listeriosis and Related Mortality Among Groups at Risk of Acquiring Listeriosis. Clinical Infectious Diseases, 54(5), pp. 652-660. doi: 10.1093/cid/cir902

Gounadaki, A.S., Skandamis, P.N., Drosinos, E.H. and Nyhas, G.E., 2008. Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. *Food Microbiology*, 25(2), pp. 313-323. doi: 10.1016/j.fm.2007.10.001

Granier, S.A., Moubareck, C., Colaneri, C., Lemire, A., Roussel, S., Dao, T.T., Courvalin, P. and Brisabois, A., 2011. Antimicrobial Resistance of *Listeria monocytogenes* Isolated from Food and the Environment in France over a 10-Year Period. *Applied and Environmental Microbiology*, 77(8), pp. 2788-2790. doi: 10.1128/AEM.01381-10

Guillet, C., Join-Lambert, O., Monnier, A.L., Leclercq, A., Mechaï, F., Mamzer-Bruneel, M.F., Bielecka, M.K., Scortti, M., Disson, O., Berche, P., Vazquez-Boland, J., Lortholary, O. and Lecuit, M., 2010. Human Listeriosis Caused by Listeria ivanovii. Emerging Infectious Diseases, 16(1), pp. 136-138. doi: 10.3201/eid1601.091155

Gupta, P., Sarkar, S., Das, B., Bhattacharjee, S. and Tribedi, P., 2016. Biofilm, pathogenesis and prevention – a journey to break the wall: a review. Archives of Microbiology, 198(1), pp. 1-15. doi: 10.1007/s00203-015-1148-6

Hafner, L., Pichon, M., Burucoa, C., Nusser, S.H.A., Moura, A., Garcera, M.G. and Lecuit, M., 2021. *Listeria monocytogenes* faecal carriage is common and depends on the gut microbiota. *Nature Communications*, 12, pp. 6826. doi: 10.1038/s41467-021-27069-y

Haley, B.J., Sonnier, J., Schukken, Y.H., Karns, J.S. and Kessel, J.A.S.V., 2015. Diversity of *Listeria monocytogenes* within a U.S. dairy herd, 2004-2010. *Foodborne Pathogens and Disease*, 12(10), pp. 844-850. doi: 10.1089/fpd.2014.1886

Harvey, J., Keenan, K.P. and Gilmour, A., 2007. Assessing biofilm formation by *Listeria monocytogenes* strains. *Food Microbiology*, 24(4), pp. 380-392. doi: 10.1016/j.fm.2006.06.006

Hassan, Z., Purwati, E., Radu, S., Rahim, R.A. and Rusul, G., 2001. Prevalence of *Listeria* spp. and *Listeria monocytogenes* in meat and fermented fish in Malaysia. *Southeast Asian Journal of tropical medicine and public health*, 32(2), pp. 402-407.

Haubert, L., Mendonça, M., Lopes, G.V., de Itapema Cardoso, M.R. and da Silva, W.P., 2015. *Listeria monocytogenes* isolates from food and food environment harbouring tetM and ermB resistance genes. *Letters in Applied Microbiology*, 62(1), pp. 23-29. doi: 10.1111/lam.12516

Herigstad, B., Hamilton, M. and Heersink, J., 2001. How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods*, 44 (2), pp. 121-129. doi: 10.1016/s0167-7012(00)00241-4

Hoelzer, K., Sauders, B.D., Sanchez, M.D., Olsen, P.T., Pickett, M.M., Mangione, K.J., Rice, D.H., Corby, J., Sitch, S., Fortes, E.D., Roof, S.E., Grohn, Y.T., Wiedmann, M. and Oliver, H.F., 2011. Prevalence, distribution, and diversity of *Listeria monocytogenes* in retail environments, focusing on small

establishments and establishments with a history of failed inspections. *Journal of Food Protection*, 74(7), pp. 1083-1095. doi: 10.4315/0362-028X.JFP-10-567

Hof, H., Lampidis, R. and Bensch, J., 2000. Nosocomial listeria gastroenteritis in a newborn, confirmed by random amplification of polymorphic DNA. *Clinical Microbiology and Infection*, 6(12), pp. 683-686. doi: 10.1046/j.1469-0691.2000.00111.x

Holah, I.T. and Thorne, R.H., 1990. Cleanability in relation on unused and abraded domestic sink materials. *Journal of Applied Bacteriology*, 69, pp. 599-608. doi: 10.1111/j.1365-2672.1990.tb01554.x

Hsih, H. and Tsen, H., 2001. Combination of Immunomagnetic Separation and Polymerase Chain Reaction for the Simultaneous Detection of *Listeria monocytogenes* and *Salmonella* spp. in Food Samples. *Journal of Food Protection*, 64(11), pp. 1744-1750. doi: 10.4315/0362-028x-64.11.1744

Huang, L. and Hwang, C., 2012. In-package pasteurization of ready-to-eat meat and poultry products. *Advances in Meat, Poultry and Seafood Packaging*, Chapter 16, pp. 437-450. doi: 10.1533/9780857095718.3.437

Hyde, F.W., Alberg, M. and Smith, K., 1997. Comparison of fluorinated polymers against stainless steel, glass and polypropylene in microbial biofilm adherence and removal. *Journal of Industrial Microbiology and Biotechnology*, 19(2), pp. 142-147. doi: 10.1038/sj.jim.2900448

Iannetti, L., Acciari, V.A., Antoci, S., Addante, N., Bardasi, L., Bilei, S., Calistri, P., Cito, F., Cogoni, P., D'Aurelio, R., Decastelli, L., Iannetti, S., Iannitto, G., Marino, A.M.F., Muliari, R., Neri, D., Perilli, M., Pomilio, F., Prencipe,V.A., Proroga, Y., Santarelli, G.A., Sericola, M., Torresi, M. and Migliorati, G., 2016. *Listeria monocytogenes* in ready-to-eat foods in Italy: Prevalence of contamination at retail and characterization of strains from meat products and cheese. *Food Control*, 68, pp. 55-61. doi: 10.1016/j.foodcont.2016.03.036

Ibusquiza, P.S., Herrera, J.J.R. and Cabo, M.L., 2011. Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by *Listeria monocytogenes*. *Food Microbiology*, 28(3), pp. 418-425. doi: 10.1016/j.fm.2010.09.014

Inoue, S., Nakama, A., Arai, Y., Kokubo, Y., Maruyama, T., Saito, A., Yoshida, T., Terao, M., Yamamoto, S. and Kumagai, S., 2000. Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan.

International Journal of Food Microbiology, 59(1-2), pp. 73-77. doi: 10.1016/S0168-1605(00)00284-1

Jacks, A., Pihlajasaari, A., Vahe, M., Myntti, A., Kaukoranta, S.S., Elomma, N., Salmenlinna, S., Rantala, L., Lahti, K., Huusko, S., Kuusi, M., Sitonen, A. and Rimhanen-Finne, R., 2015. Outbreak of hospital-acquired gastroenteritis and invasive infection caused by *Listeria monocytogenes*, Finland, 2012. *Epidemiology and Infection*, 144(13), pp. 2732-2742. doi: 10.1017/S0950268815002563

Jallewar, P.K., Kalorey, D.R., Kurkure, N.V., Pande, V.V. and Barbuddhe, S.B., 2007. Genotypic characterization of *Listeria* spp. isolated from fresh water fish. *International Journal of Food Microbiology*, 114(1), pp. 120-123. doi: 10.1016/j.ijfoodmicro.2006.09.034

Jamali, H., Chai, L.C. and Thong, K.L., 2013. Detection and isolation of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. *Food Control*, 32(1), pp. 19-24. doi: 10.1016/j.foodcont.2012.11.033

James, N.R. and Jayakrishnan, A., 2003. Surface thiocyanation of plasticized poly (vinyl chloride) and its effect on bacterial adhesion. *Biomaterials*, 24(13), pp. 2205-2212. doi: 10.1016/s0142-9612(03)00022-x

Jemmi, T. and Stephan, R., 2006. *Listeria monocytogenes*: food-borne pathogen and hygiene indicator. *Revue Scientifique et Technique (International Office of Epizootics)*, 25 (2), pp. 571-580. PMID: 17094698.

Jeyaletchumi, P., Tunung, R., Margaret, S.P., Son, R., Farinazleen, M.G. and Cheah, Y.K., 2010. Review Article: Detection of *Listeria monocytogenes* in foods. *International Food Research Journal*, 17, pp. 1-11.

Kabwanga, I.T., Yetişemiyen, A. and Nankaya, S., 2018. Dairy Industrial Hygiene: A review on biofilm challenges and control. *International Journal of Research Granthaalayah*, 6(2), pp. 268-273. doi: 10.29121/granthaalayah.v6.i2.2018.1570

Kadam, S.R., den Besten, H.M., van der Veen, S., Zwietering M.H., Moezelaar, R. and Abee, T., 2013. Diversity assessment of *Listeria monocytogenes* biofilm formation: impact of growth condition, serotype and strain origin. *International Journal of Food Microbiology*, 165(3), pp. 259-264. doi: 10.1016/j.ijfoodmicro.2013.05.025

Kalender, H., 2011. Prevalence of *Listeria* species in ground beef and chicken meat sold in Eastern Turkey. *Pakistan Veterinary Journal*, 32(3), pp. 456-458.

Kataoka, A., Wang, H., Elliott, P.H., Whiting, R.C. and Hayman, M.M., 2017. Growth of *Listeria monocytogenes* in Thawed Frozen Foods. *Journal of Food Protection*, 80 (3), pp. 447-453. doi: 10.4315/0362-028X.JFP-16-397R

Kathariou, S., 2002. *Listeria monocytogenes* Virulence and Pathogenicity, a Food Safety Perspective. *Journal of Food Protection*, 65(11), pp. 1811-1829. doi: 10.4315/0362-028x-65.11.1811

Kaur, M. (2019). *Food poisoning cases up to by 24% including in schools, reveals Dzulkefly*, [online] Available at: <https://www.freemalaysiatoday.com/category/nation/2019/07/13/food-poisoning-cases-up-by-24-including-in-schools-reveals-dzulkefly/> [Accessed 6 December 2021].

Kawakami, H., Kittaka, K., Sato, Y. and Kikuchi, Y., 2010. Bacterial Attachment and Initiation of Biofilms on the Surface of Copper Containing Stainless Steel. *ISIJ International*, 50(1), pp. 133-138. doi: 10.2355/isijinternational.50.133

Khan, J.A., Rathore, R.S. and Ahmad, I., 2013. In vitro detection of pathogenic *Listeria monocytogenes* from food sources by conventional, molecular and cell culture method. *Brazilian Journal of Microbiology*, 44(3), pp. 751-758. doi: 10.1590/s1517-83822013000300013

Khelissa, S.O., Abdallah, M., Jama, C., Faille, C. and Chihib, N.E., 2017. Bacterial contamination and biofilm formation on abiotic surfaces and strategies to overcome their persistence. *Journal of Materials and Environmental Sciences*, 8(9), pp. 3326-3346.

Kevenk, T.O. and Gulel, G.T., 2015. Prevalence, Antimicrobial Resistance and Serotype Distribution of *Listeria monocytogenes* Isolated from Raw Milk and Dairy Products. *Journal of Food Safety*, 36(1), pp. 11-18. doi: 10.1111/jfs.12208

Kuan, C.H., Goh, S.G., Loo, Y.Y., Chang, W.S., Lye, Y.L., Soopna, P., Tang, J.Y.H., Nakaguchi, Y., Nishibuchi, M., Mahyudin, N.A. and Radu, S., 2013a. Prevalence and quantification of *Listeria monocytogenes* in chicken offal at the retail level in Malaysia. Poultry Science, 92(6), pp. 1664-1669. doi: 10.3382/ps.2012-02974

Kuan, C.H., Wong, W.C., Pui, C.F., Tang, J.Y.H., Nishibuchi, M., Mahyudin, N.A. and Radu, S., 2013b. Prevalence and quantification of *Listeria monocytogenes* in beef offal at retail level in Selangor, Malaysia. *Brazilian Journal of Microbiology*, 44(4), pp. 1169-1172. doi: 10.1590/S1517-83822014005000002

Kuan, C.H., Goh, S.G., Loo, Y.Y., Chang, W.S., Lye, Y.L., Puspanandan, S., Shahril, N., Tang, J.Y.H., Mahyudin, N.A., Nishibuchi, M., Nakaguchi, Y. and Son, R., 2015. Risk assessment of acquiring listeriosis from consumption of chicken offal in Selangor, Malaysia. *International Food Research Journal*, 22(4), pp. 1711-1718.

Kuan, C.H., Rukayadi, Y., Ahmad, S.H., Wan Mohamed Radzi, C.W.J., Kuan, C.S., Yeo, S.K., Thung, T.Y., New, C.Y., Chang, W.S., Loo, Y.Y., Tan, C.W., Ramzi, O.S.B., Mohd Fadzil, S.N., Nordin, Y., Kwan, S.Y. and Radu, S., 2017. Antimicrobial resistance of *Listeria monocytogenes* and *Salmonella Enteritidis* isolated from vegetables farms and retail markets in Malaysia. *International Food Research Journal*, 24(4), pp. 1831-1839.

Lakicevic, B., and Nastasijevic, I., 2016. *Listeria monocytogenes* in retail establishments: Contamination routes and control strategies. *Food Reviews International*, 33(3), pp. 247-269. doi: 10.1080/87559129.2016.1175017

Lakicevic, B., Biase, D.D., Velebit, B., Mitrovic, R., Jankonic, V., Milojevic, L. and Ruppitsch, W., 2021. Stress survival islets contribute to clonal and serotype-specific differences in *L. monocytogenes*. *IOP Conference Series: Earth and Environmental Science*, pp. 854. doi:10.1088/1755-1315/854/1/012050

Leong, D., Alvarez-Ordóñez, A. and Jordan, K., 2014. Monitoring occurrence and persistence of *Listeria monocytogenes* in foods and food processing environments in the Republic of Ireland. *Frontiers in Microbiology*, 5, pp. 436. doi:10.3389/fmicb.2014.00436

Leclercq, A., Moura, A., Vales, G., Tessaud-Rita, N., Aguilhon, C. and Lecuit, M., 2019. *Listeria thailandensis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 69(1), pp. 74-81. doi:10.1099/ijsem.0.003097

Lee, B.H., Cole, S., Badel-Berchoux, S., Guillier, L., Felix, B., Kredorn, N., Hébraud, M., Bernardi, T., Sultan, I. and Piveteau, P., 2019. Biofilm Formation of *Listeria monocytogenes* Strains Under Food Processing Environments and

Pan-Genome-Wide Association Study. *Frontier in Microbiology*, 10. doi: 10.3389/fmicb.2019.02698

Lee, H.K., Abdul Halim, H., Thong, K.L., Chai, L.C., 2017. Assessment of food safety knowledge, attitude and self-reported practices, and microbiological hand hygiene of food handlers. *International Journal of Environmental Research and Public Health*, 14(1), pp. 55. doi: 10.3390/ijerph14010055

Lee, J., Jeong, K. and Kim, Y., 2012. Purification and Structural Characterization of Cold Shock Protein from *Listeria monocytogenes*. *Bulletin of Korean Chemical Society*, 33(8), pp. 2508-2512. doi: 10.5012/bkcs.2012.33.8.2508

Leimesiter-Wächter, M. and Chakraborty, T., 1989. Detection of listeriolysin, the thiol-dependent hemolysin in *Listeria monocytogenes*, *Listeria ivanovii*, and *Listeria seeligeri*. *Infection and Immunity*, 57(8), pp. 2350-2357. doi: 10.1128/iai.57.8.2350-2357.1989

Li, X., Boudjellab, N. and Zhao, X., 2000. Combined PCR and slot blot assay for detection of *Salmonella* and *Listeria monocytogenes*. *International Journal of Food Microbiology*, 56(2-3), pp. 167-177. doi: 10.1016/s0168-1605(99)00209-3

Lihan and Samuel., 2007. *Prevalence and characterization of Listeria monocytogenes isolated from chicken and beef. PHD thesis, Universiti Putra Malaysia.* [online] Available at: <http://psasir.upm.edu.my/id/eprint/5321/> [Accessed 13 February 2013].

Linke, K., Rückerl, I, Brugger, K., Karpiskova, R., Walland, J., Muri-Klinger, S., Tichy, A., Wagner, M. and Stessl, B., 2014. Reservoirs of *Listeria* species in three environmental ecosystems. *Applied and Environmental Microbiology*, 80, pp. 5583-5592. doi: 10.1128/AEM.01018-14

Lippert, K. and Galinski, E.A., 1992. Enzyme stabilization by ectoine-type compatible solutes: protection against heating, freezing and drying. *Applied and Microbiology Biotechnology*, 37(1), pp. 61-65. doi:10.1007/BF00174204

Liu, D., Lawrence, M.L., Ainsworth, A.J. and Austin, F.W., 2005. Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. *Federation of European Microbiological Societies Microbiology Letters*, 243 (2), pp. 373-378. doi: 10.1016/j.femsle.2004.12.025

Liu, D., 2006. Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. *Journal of Medical Microbiology*, 55(6), pp. 645-659. doi: 10.1099/jmm.0.46495-0

Liu, D., Lawrence, M.L., Austin, F.W. and Ainsworth, A.J., 2007. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. *Journal of Microbiological Methods*, 71(2), pp. 133-140. doi: 10.1016/j.mimet.2007.08.007

Londero, A., Costa, M., Sucari, A. and Leotta, G., 2019. Comparison of three molecular subtyping techniques for *Listeria monocytogenes*. *Revista Argentina de Microbiología*, 51(4), pp. 359-362. doi: 10.1016/j.ram.2019.01.003

Lundén, J.M., Miettinen, M.K., Autio, T.J. and Korkeala, H.J., 2000. Persistent *Listeria monocytogenes* Strains Show Enhanced Adherence to Food Contact Surfaces after Short Contact Time. *Journal of Food Protection*, 63(9), pp. 1204-1207. doi: 10.4315/0362-028x-63.9.1204

Lundén, J., Autio, T., Markkula, A., Hellström, S. and Korkeala, H., 2003. Adaptive and cross- adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. *International Journal of Food Microbiology*, 82, pp. 265-272. doi: 10.1016/s0168-1605(02)00312-4

Lyautey, E., Hartamann, A., Pagotto, F., Tyler, K., Lapen, D.R., Wilkes, G., Piveteau, P., Rieu, A., Robertson, W.J., Medeiros, D.T., Edge, T.A., Gannon, V. and Topp, E., 2007. Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of south nation river watershed, Ontario, Canada. *Applied Environment Microbiology*, 73(5), pp. 401-410. doi: 10.1128/AEM.00354-07

Mafu, A.A., Roy, D., Goulet, J. and Magny, P., 1990. Attachment of *Listeria monocytogenes* to stainless steel, glass, polypropylene and rubber surfaces after short contact time. *Journal of Food Protection*, 53(9), pp. 742-746. doi: 10.4315/0362-028X-53.9.742

Maillard, J.Y., 2002. Bacterial target sites for biocide action. *Journal of Applied Microbiology*, 92(1), pp. 16S-27S. PMID: 12000609

Malay Mail, 2015. *Listeriosis: Identifying A Potential Killer*. Mediaworks Malaysia., p.1.

Manfreda, G., Cesare, A.D., Stella, S., Cozzi, M. and Cantoni, C., 2005. Occurrence and ribotypes of *Listeria monocytogenes* in Gorgonzola cheeses. *International Journal of Food Microbiology*, 102(3), pp. 287-293. doi: 10.1016/j.ijfoodmicro.2004.11.045

Marian, A.R., Ismail, M., Nurilyana, M.T., Mohd Fharok, Y. and Azzura, A.L., 2019. Incidence of *Listeria monocytogenes* in dairy and food products of animal origin in central region of Peninsular Malaysia. *Malaysian Journal of Veterinary Research*, 10(2), pp. 106-112

Marian, M.N., Sharifah Aminah, S.M., Zuraini, M.I., Radu, S., Maimunah, M., Lee, H.Y., Wong, W.C. and Elexson, N., 2012. MPN-PCR detection and antimicrobial resistance of *Listeria monocytogenes* isolated from raw and ready-to-eat foods in Malaysia. *Food Control*, 28(2), pp. 309-314. doi: 10.1016/j.foodcont.2012.05.030

Martín, B., Jofré, A., Garriga, M., Hugas, M. and Aymerich, T., 2004. Quantification of *Listeria monocytogenes* in fermented sausages by MPN-PCR method. *Letters in Applied Microbiology*, 39(3), pp. 290-295. doi: 10.1111/j.1472-765X.2004.01580.x

Meloni, D., Galluzzo, P., Mureddu, A., Piras, F., Griffiths, M. and Mazzette, R., 2009. *Listeria monocytogenes* in RTE Foods Marketed in Italy: Prevalence and Automated EcoRI Ribotyping of the Isolates. *International Journal of Food Microbiology*, 129(2), pp. 166-173. doi: 10.1016/j.ijfoodmicro.2008.11.014

Mhurchu, C.N., Capelin, C., Dunford, E.K., Webster, J.L., Neal, B.C. and Jebb, S.A., 2010. Sodium content of processed foods in United Kingdom: analysis of 44,000 foods purchased by 21,000 households. *American Journal of Clinical Nutrition*, 93(3), pp. 594-600. doi: 10.3945/ajcn.110.004481

Midelet, G., Kobilinsky, A. and Carpentier, B., 2006. Construction and analysis of fractional multifactorial designs to study attachment strength and transfer of *Listeria monocytogenes* from pure or mixed biofilms after contact with a solid model food. *Applied and Environmental Microbiology*, 72, pp. 2313-2321. doi: 10.1128/AEM.72.4.2313-2321.2006

Miettienen, M.K., Siitonenm A., Heiskanen, P., Haajanen, H., Björkroth, K.J. and Korkeala, H.J., 1999. Molecular Epidemiology of an Outbreak of Febrile Gastroenteritis Caused by *Listeria monocytogenes* in Cold-Smoked Rainbow Trout. *Journal of Clinical Microbiology*, 37(7), pp. 2358-2360. PMID:10364616.

Miladi, H., Ammar, E., Ben Slama, R., Sakly, N. and Bakhrouf, A., 2013. Influence of freezing stress on morphological and membrane fluidity. *Archives of Microbiology*, 195(10-11), pp. 705-715. doi: 10.1007/s00203-013-0921-7

Ministry of Agriculture and Forestry (MAF)., 2011. *Guidance for the control of Listeria monocytogenes in ready-to-eat foods.* [online] Available at:<http://www.higieneambiental.com/sites/default/files/images/pdf/guidance-listeria-monocytogenes.pdf> [Accessed 3 February 2023].

Monteiro, C.A., Cannon, G., Levy, R., Moubarac, J., Jaime, P., Martins, A.P., Canella, D., Louzada, M., Parra, D., Ricardo, C., Calixto, G., Machado, P., Martins, C., Martinez, E., Baraldi, L., Garzillo, J. and Sattamini, I., 2016. Food classification. Public health NOVA. The star shines bright. *World Nutrition*, 7(1-3), pp. 28-38.

Morvan, A., Moubareck, C., Leclercq, A., Hervé-Bazin, M., Bremot, S., Lecuit, M., Courvalin, P. and Le Monnier, A., 2020. Antimicrobial Resistance of *Listeria monocytogenes* Strains Isolated from Human in France. *American Society for Microbiology Antimicrobial Agents and Chemotherapy*, 54(6), pp. 2728-2731. doi: 10.1128/AAC.01557-09

Mpundu, P., Mbewe, A.R., Muma, J.B., Zgambo, J. and Munyeme, M., 2019. Evaluation of bacterial contamination in dressed chickens in Lusaka Abattoirs. *Frontiers in Public Health*, 7. doi: 10.3389/fpubh.2019.00019

Muchaamba, F., Eshwar, A.K., Stevens, M.J.A., Stephan, R. and Tasara, T., 2022. Different Shades of *Listeria monocytogenes*: Strain, Serotype, and Lineage-Based Variability in Virulence and Stress Tolerance Profiles. *Frontiers in Microbiology*, 12. doi: doi.org/10.3389/fmicb.2021.792162

Muhammad, M.H., Idris, A.L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X. and Huang, T., 2020. Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. *Frontiers Microbiology*, 11. doi: 10.3389/fmicb.2020.00928

Müller, A., Rychli, K., Zaiser, A., Wieser, C., Wagner, M. and Schmitz-Esser, S., 2014. The *Listeria monocytogenes* transposon Tn6188 provides increased tolerance to various quaternary ammonium compounds and ethidium bromide. *FEMS Microbiology Letters*, 361(2), pp. 166-173. doi: 10.1111/1574-6968.12626

Mureddu, A., Mazza, R., Fois, F., Meloni, D., Bacciu, R., Piras, F. and Mazzette, R., 2014. *Listeria monocytogenes* persistence in ready-to-eat sausages and in

processing plants. *Italian Journal of Food Safety*, 3(1), pp. 1697. doi: 10.4081/ijfs.2014.1697

Nadon, C.A., Woodward, D.L., Young, C., Rodgers, F.G. and Wiedmann, M., 2001. Correlations between Molecular Subtyping and Serotyping of *Listeria monocytogenes*. *Journal of Clinical Microbiology*, 39(7), pp. 2704-2707. doi: 10.1128/JCM.39.7.2704-2707.2001

Nayak, D.N., Savalia, C.V., Kalyani, I.H., Kumar, R. and Kshirsagar, D.P., 2015. Isolation, identification and characterization of *Listeria* spp. from various animal origin foods. *Veterinary World*, 8(6), pp. 695-701. doi: 10.14202/vetworld.2015.695-701

Ndahi, M.D., Kwaga, J.K.P., Bello, M., Kabir, J., Umoh, V.J., Yakubu, S.E. and Nok, A.J., 2013. Prevalence and antimicrobial susceptibility of *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus* strains from raw meat and meat products in Zaria, Nigeria. *Letters in Applied Microbiology*, 58(3), pp. 262-269. doi: 10.1111/lam.12183

Neunlist, M.R., Federighi, M., Laroche, M., Sohier, D., Delattre, G., Jacquet, C. and Chihib, N.E., 2005. Cellular lipid fatty acid pattern heterogeneity between reference and recent food isolates of *Listeria monocytogenes* as a response to cold stress. *Antonie, Van Leeuwenhoek*, 88(3-4), pp. 199-206. doi: 10.1007/s10482-005-5412-7

Nilsson, R.E., Ross, T. and Bowman, J.P., 2011. Variability in biofilm production by *Listeria monocytogenes* correlated to strain origin and growth conditions. *International Journal of Food Microbiology*, 150(1), pp. 14-24. doi: 10.1016/j.ijfoodmicro.2011.07.012

Norwood, D.E. and Gilmour, A., 2001. The differential adherence capabilities of two *Listeria monocytogenes* strains in monoculture and multispecies biofilms as a function of temperature. *Letters in Applied Microbiology*, 33(4), pp. 320-324. doi: 10.1046/j.1472-765x.2001.01004.x.

Nyfeldt, A., 1929. Étiologie de la mononucléose infectieuse. *Comptes Rendus des Séances de la Societé de Biologie*, 101, pp. 590-591.

Nwaiwu, O., 2020. What are the recognized species of the genus *Listeria*? *Access Microbiology*, 2(9). doi: 10.1099/acmi.0.000153

Olaimat, A.N., Al-Holy, M.A., Shahbaz, H.M., Al-Nabulsi, A.A., Ghoush, M.H.A., Osaili, T.M., Ayyash, M.M. and Holley, R.A., 2018. Emergence of Antibiotic Resistance in *Listeria monocytogenes* Isolated from Food Products: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, 17(5), pp. 1277-1292. doi: 10.1111/1541-4337.12387

Oliveira, M.M.M., Brugnera, D.F., Cardoso, M.G., Alves, E. and Piccoli, R.H., 2010. Disinfectant action of Cymbopogon sp. Essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. *Food Control*, 21(4), pp. 549-553. doi: 10.1016/j.foodcont.2009.08.003

Ooi, S.T. and Lorber, B., 2005. Gastroenteritis Due to *Listeria monocytogenes*. *Clinical Infectious Diseases*, 40(9), pp. 1327-1332. doi: 10.1086/429324

Orsi, R. H., Bakker, H. C. D., and Wiedmann, M., 2011. *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. *International Journal of Medical and Microbiology*, 301, pp. 79–96. doi: 10.1016/j.ijmm.2010.05.002

Orsi, R.H. and Wiedmann, M., 2016. Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Applied Microbiology and Biotechnology*, 2016 (100), pp. 5273-5287. doi: 10.1007/s00253-016-7552-2

Osaili, T.M., Alaboudi, A.R. and Nesiar, E.A., 2011. Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. *Food Control*, 22 (3-4), pp. 586-590. doi: 10.1016/j.foodcont.2010.10.008

Osman, K., Kappell, A.D., Fox, E.M., Orabi, A. and Samir, A., 2020. Prevalence, Pathogenicity, Virulence, Antibiotic Resistance, and Phylogenetic Analysis of Biofilm-Producing *Listeria monocytogenes* Isolated from Different Ecological Niches in Egypt: Food, Humans, Animals and Environment. *Pathogens*, 9(1), pp. 5. doi: 10.3390/pathogens9010005

Pagatto, F.J. and Farber, J.M., 2003. *Listeria/listeriosis*. In: Caballero, B., Trugo, L. and Finglas, P. (eds), Encyclopedia of Food Sciences and Nutrition, pp. 3582-3588. Academic Press, London.

Palumbo, J.D., Borucki, M.K., Mandrell, R.E. and Gorski, L., 2003. Serotyping of *Listeria monocytogenes* by Enzyme-Linked Immunosorbent Assay and Identification of Mixed-Serotype Cultures by Colony Immunoblotting. *Journal*

of Clinical Microbiology, 41(2), pp. 564-571. doi: 10.1128/JCM.41.2.564-571.2003

Pan, Y., Breidt, F. and Gorski, L., 2010. Synergistic effects of sodium chloride, glucose, and temperature on biofilm formation by *Listeria monocytogenes* serotype 1/2a and 4b strains. *Applied and Environmental Microbiology*, 76(5), pp. 1433-1441. doi: 10.1128/AEM.02185-09

Paramithiotis, S., Kotsakou, C. and Drosinos, E.H., 2021. Transcription of *Listeria monocytogenes* Key Virulence Genes on Tomato, Cucumber and Carrot. *Applied Sciences*, 11(13), pp. 5983. doi: 10.3390/app11135983

Pérez-Ibarreche, M., Castellano, P., Leclercq, A. and Vignolo, G., 2016. Control of *Listeria monocytogenes* biofilms on industrial surfaces by bacteriocin-producing *Lactobacillus sakei* CRL1862. *FEMS Microbiology Letters*, 363(12). doi: 10.1093/femsle/fnw118

Pesavento, G., Ducci, B., Nieri, D., Comodo, N. and Lo Nostro, A., 2010. Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. *Food Control*, 21(5), pp. 708-713. doi: 10.1016/j.foodcont.2009.10.012

Piercey, M.J., Hingston, P.A and Truelstrup Hansen, L., 2016. Genes involved in *Listeria monocytogenes* biofilm formation at a simulated food processing plant temperature of 15ºC. *International Journal of Food Microbiology*, 223, pp. 63-74. doi: 10.1016/j.ijfoodmicro.2016.02.009

Ponniah, J., Robin, T., Paie, M.S., Radu, S., Ghazali, F.M., Kqueen, C.Y., Nishibuchi, M., Nakaguchi, Y.and Malakar, P.K., 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. *Food Control*, 21(5), pp. 774-778. doi: 10.1016/j.foodcont.2009.09.008

Pontello, M., Guaita, A., Sala, G., Cipolla, M., Gattuso, A., Sonnessa, M. and Gianfranceschi, M.V., 2012. *Listeria monocytogenes* serotypes in human infections (Italy, 2000-2010). *Annali dell'lstituto superior di sanita*, 48(2), pp. 146-150.doi: 10.4415/ANN_12_02_07

Poti, J.M., Mendez, M.A., Ng, S.W. and Popkin, B.M., 2015. Is the degree of food processing and convenience linked with the nutritional quality of foods purchased by US households? *The American Journal of Clinical Nutrition*, 101(6), pp. 1251-1262. doi: 10.3945/ajcn.114.100925

Poti, J.M., Mendez, M.A., Ng, S.W. and Popkin, B.M., 2016. Highly Processed and Ready-to-Eat Packaged Food and Beverage Purchases Differ by Race/Ethnicity among US Households. *The Journal of Nutrition*, 146(9), pp. 1722-1730. doi: 10.3945/jn.116.230441

Public Health England., 2017. *Detection and Enumeration of Bacteria in Swabs and Other Environmental Samples. National Infection Service Food Water and Environmental Microbiology Standard Method*, [online] Available at: <https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/660648/Detection_and_enumeration_of_bacteria_in_swabs_and_other_environmental_samples.pdf> [Accessed on 5 April 2020]

Pyz-Łukasik, Paszkiewicz, Kielbus, M., Ziomek, M., Gondek, M., Domaradzki, P., Michalak, K. and Pietras-Ożga, D., 2022. Genetic Diversity and Potential Virulence of *Listeria monocytogenes* Isolates Originating from Polish Artisanal Cheeses. *Foods*, 11(18), pp. 2805. doi: 10.3390/foods11182805

Quereda, J.J., Morón-García, A., Palacios-Gorba, C., Dessaux, C., Portillo, F.G., Pucciarelli, M.G. and Ortega, A.D., 2021. Pathogenicity and virulence of *Listeria monocytogenes*: A trip from environmental to medical microbiology. *Virulence*, 12(1), pp. 2509-2545. doi: 10.1080/21505594.2021.1975526

Ranjbar, R. and Halaji, M., 2018. Epidemiology of *Listeria monocytogenes* prevalence in foods, animals and human origin from Iran: a systematic review and meta-analysis. *BMC Public Health*, 18, pp. 1057. doi: 10.1186/s12889-018-5966-8

Roberts, A., Nightingale, K., Jeffers, G., Fortes, E., Kongo, J.M. and Wiedmann, M., 2006. Genetic and Phenotypic Characterization of *Listeria monocytogenes* lineage III. *Microbiology Society*, 152(3), pp. 685-693. doi: 10.1099/mic.0.28503-0

Radoshevich, L. and Cassart, P. *Listeria monocytogenes*: towards a complete picture of its physiology and pathogenesis. *Nature Reviews Microbiology*, 16(1), pp. 32-46. doi: 10.1038/nrmicro.2017.126

Rodríguez-López, P., Rodríguez-Herrera, J.J., Vázquez-Sánchez, D. and Cabo, M.L., 2018. Current Knowledge on *Listeria monocytogenes* Biofilms in Food-Related Environments: Incidence, Resistance to Biocides, Ecology and Biocontrol. *Foods*, 7(6), E85. doi: 10.3390/foods/7060085

Rolfe, C. and Daryaei, H., 2020. Intrinsic and Extrinsic Factors Affecting Microbial Growth in Food Systems. Food Safety Engineering. *Food Engineering Series*. *Springer*, Cham, pp. 3-24.doi:10.1007/978-3-030-42660-6_1

Romanova, N.A., Wolffs, P.F.G., Brovko, L.Y. and Griffiths, M.W., 2006. Role of efflux pumps in adaptation and resistance of *Listeria monocytogenes* to benzalkonium chloride. *Applied and Environmental Microbiology*, 72(5), pp. 3498-3503. doi: 10.1128/AEM.72.5.3498-3503.2006

Rawool, D.B., Malik, S.V.S., Barbuddhe, S.B., Shakuntala, I. and Aurora, R., 2007. A Multiplex PCR for Detection of Virulence Associated Genes in *Listeria monocytogenes*. *Internet Journal of Food Safety*, 9, pp. 56-62. doi: 10.1.1.532.4177

Sabet, C., Lecuit, M., Cabanes, D., Cossart, P. and Bierne, H., 2005. LPXTG Protein *Inl*J, a Newly Identified Internalin Involved in *Listeria monocytogenes* Virulence. *Infection and Immunity*, 73(10), pp. 6912-6922. doi: 10.1128/IAI.73.10.6912-6922.2005

Sauders, B.D., Pettit, D., Currie, B., Suits, P. Evans, A., Stellrecht, K., Dryja, D.M., Slate, D. and Wiedmann, M., 2005. Low Prevalence of *Listeria monocytogenes* in Human Stool. *Journal of Food Protection*, 68(1), pp. 178-181

Sauders, B.D., Overdevest, J., Fortes, E., Windham, K., Schukken, Y., Lembo, A. and Wiedmann, M., 2012. Diversity of *Listeria* species in urban and natural environments. *Applied and Environmental of Microbiology*, 78(12), pp.4420-4433. doi: 10.1128/AEM.00282-12

Schäfer, D.F., Steffens. J., Barbosa, J., Zeni, J., Paroul, N., Valduga, E., Junges, A., Backes, G.T. and Cansian, R.L., 2017. Monitoring of contamination sources of *Listeria monocytogenes* in a poultry slaughterhouse. *LWT – Food Science and Technology*, 86, pp. 393-398. doi: 10.1016/j.lwt.2017.08.024

Schlech III, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholas, E.S. and Broome, C.V., 1983. Epidemic Listeriosis – Evidence for Transmission by Food. *The New England Journal of Medicine*, 308(4), pp. 203-206. doi: 10.1056/NEJM198301273080407

Schmid, B., Klumpp J., Raimann, E., Loessner, M.J., Stephan, R. and Tasara, T., 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under

cold and osmotic stress conditions. *Applied and Environmental Microbiology*, 75(6), pp. 1621-1627. doi: 10.1128/AEM.02154-08

Scollon, A.M., Wang, H. and Ryser, E.T., 2016. Transfer of *Listeria monocytogenes* during mechanical slicing of onions. *Food Control*, 65, pp. 160-167. doi: 10.1016/j.foodcont.2016.01.021

Shapiro, R.S., 2015. Antimicrobial-Induced DNA Damage and Genomic Instability in Microbial Pathogens. *PLoS Pathogenesis*, 11(3), e1004678. doi: 10.1371/journal.ppat.1004678.

Silk, B.J., Date, K.A., Jackson, K.A., Pouillot, R., Holt, K.G., Graves, L.M., Ong, K.L., Hurd, S., Meyer, R., Marcus, R., Shiferaw, B., Norton, D.M., Medus, C., Zansky, S.M., Cronquist, A.B., Henao, O.L., Jones, T.F., Vugia, D.J., Farley, M.M. and Mahon, B.E., 2012. Invasive listeriosis in the foodborne diseases active surveillance network (FoodNet), 2004-2009: Further targeted prevention needed for higher-risk groups. *Clinical Infectious Diseases*, 54(5), pp. 396-404. doi: 10.1093/cid/cis268

Skowron, K., Walecka-Zacharska, E., Grudlewska, K., Gajewski, P., Wiktorczyk, N., Wietlicka-Piszcz, M., Dudek, A., Skowron, K.J. and Gospodarek-Komkowska, E., 2019. Disinfectant Susceptibility of Biofilm Formed by *Listeria monocytogenes* under selected Environmental Conditions. *Microorganisms*, 7(9), pp. 280. doi: 10.3390/microorganisms7090280

Smoot, L.M. and Pierson, M.D., 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. *Journal of Protection*, 61(10), pp. 1293-1298. doi: 10.4315/0362-028x-61.10.1293

Snide, E. and Carballo, J., 2000. Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluro ethylene: the influence of free energy and the effect of commercial sanitizers. *Food Microbiology*, 17(4), pp. 439-447. doi: 10.1006/fmic.2000.0339

Soni, D.K. and Dubey, S.K., 2014. Phylogenetic analysis of the *Listeria monocytogenes* based on the sequencing of 16S rRNA and hly*A* genes. *Molecular Biology Reports*, 41(2), pp. 8219-8229. doi: 10.1007/s11033-014-3724-2

Soni, D.K., Singh, M., Singh, D.V. and Dubey, S.K. (2014). Virulence and genotypic characterization of Listeria monocytogenes isolated from vegetable

and soil samples. BMC Microbiology, 14(1), pp. 241. doi: 10.1186/s12866-014-0241-3

Swaminathan, B., Cabanes, D., Zhang, W., Cossart, P., 2007. *Listeria monocytogenes*, Chapter 21, pp. 457–491. In: Doyle, M.P., Beuchat, L.R. (Eds.), *Food Microbiology*: Fundamentals and Frontiers, third ed. American Society for Microbiology Press, Washington D.C.

Szymczack, B., Szymack, M., Sawicki, W. and Dabrowski, W., 2014. Anthropogenic impact on the presence of *Listeria monocytogenes* in soil, fruits and vegetables. *Folia Microbiologica*, 59(1), pp. 23-29. doi: 10.1007/s12223-013-0260-8

Tapia de Daza, M.S., Villegas, Y. and Martínez, A.J., 1991. Minimal water activity for growth of *Listeria monocytogenes* as affected by solute and temperature. *International Journal of Food Microbiology*, 14 (3-4), pp. 333-337. doi: 10.1016/0168-1605(91)90125-9

Tang, M.Y., Cheong, Y.M. and Zainuldin, T., 1994. Incidence of *Listeria* spp. in vegetables in Kuala Lumpur. *Medical Journal of Malaysia*, 49(3), pp. 217-222. PMID: 7845269.

Thévenot, D., Delignette-Muller, M., Christieans, S. and Vernozy-Rozand, C., 2005. Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. *International Journal of Food Microbiology*, 102(1), pp. 85-94. doi: 10.1016/j.ijfoodmicro.2004.12.008

Tomičić, R.M., Čabarkapa, I.S., Vukmirović, D.M., Lević, J.D. and Tomičić, Z.M., 2016. Influence of growth conditions on biofilm formation of *Listeria monocytogenes*. *Food and Feed Research*, 43(1), pp. 19-24. doi: 10.5937/FFR1601019T

Toomey, N., Monaghan, A., Fanning, S. and Bolton, D.J., 2009. Assessment of antimicrobial resistance transfer between lactic acid bacteria and potential foodborne pathogens using in vitro methods and mating in a food matrix. *Foodborne Pathogens and Disease*, 6(8), pp. 925-933. doi: 10.1089/fpd.2009.0278

USDA Agricultural Marketing Service., 2008. *Country of Origin Labelling (COOL) Frequently Asked Questions.* [online] Available at: <https://www.ams.usda.gov/sites/default/files/media/FAQs%20for%20Consumers%20-%20English.pdf> [Accessed 20 November 2022].

Vaid, R., Linton, R.H. and Morgan, M.T., 2010. Comparison of inactivation of *Listeria monocytogenes* within a biofilm matrix using chlorine dioxide gas, aqueous chlorine dioxide and sodium hypochlorite treatments. *Food Microbiology*, 27(8), pp. 979-984. doi: 10.1016/j.fm.2010.05.024

Valderrama, W.B., Mills, E.W. and Cutter, C.N., 2009. Efficacy of chlorine dioxide against *Listeria monocytogenes* in brine chilling solutions. *Journal of Food Protection*, 72(11), pp. 2272-2277. doi: 10.4315/0362-028x-72.11.2272

Van, D., 2020. *Cooking Meat? Check the New Recommended Temperatures*. [online] Available at: <https://www.usda.gov/media/blog/2011/05/25/cooking-meat-check-new-recommended-temperatures> [Accessed 15 December 2022].

Van der Veen, S. and Abee, T., 2011. Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. *International Journal of Food Microbiology*, 144(3), pp. 421-431. doi: 10.1016/j.ijfoodmicro.2010.10.029

Vasconcelos, R. M. de., Almeida, A. E. C. C. de., Hofer, E., Silva, N. M. M. da., and Marin, V. A., 2008. Multiplex-PCR serotyping of *Listeria monocytogenes* isolated from human clinical specimens. *Memórias Do Instituto Oswaldo Cruz*, 103(8), pp. 836–838. doi: 10.1590/S0074-02762008000800016

Vazquez-Boland, J.A., Kuhn, M., Beche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J. and Kreft, J., 2001. *Listeria* Pathogenesis and Molecular Virulence Determinants. *Clinical Microbiology Reviews*, 14 (3), pp. 584-640. doi: 10.1128/CMR.14.3.584-640.2001

Vitas, A.I. and Garcia-Jalon, V.A.eI., 2004. Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). International *Journal of Food Microbiology*, 90(3), pp. 349-356. doi: 10.1016/S0168-1605(03)00314-3

Vivant, A.L., Garmyn, D. and Piveteau, P., 2013. *Listeria monocytogenes*, a down to earth pathogen. *Frontiers in Cellular and Infection Microbiology*, 28(3), pp. 87. doi: 10.3389/fcimb.2013.00087

Wang, W., Zhou, X., Suo, Y., Deng, X., Cheng, M., Shi, C. and Shi, X., 2017. Prevalence, serotype diversity, biofilm-forming ability and eradication of *Listeria monocytogenes* isolated from diverse foods in Shanghai, China. *Food Control*, 73, pp. 1068-1073. doi: 10.1016/j.foodcont.2016.10.025

Webster, J.L., Dunford, E.K. and Neal, B.C., 2009. A systematic survey of the sodium contents of processed foods. The American Journal of Clinical Nutrition, 91 (2), pp. 413-420. doi: 10.3945/ajcn.2009.28688

Wei, C.I, Cook, D.L. and Kirk, J.R., 1985. Use of chlorine compounds in the food industry. *Food Technology*, 39, pp. 107-115.

Wiedmann, M.., Bruce, J.L., Knorr, R., Bodis, M., Cole, E.M., McDowell, C.I., McDonough, P.L. and Batt, C.A., 1996. Ribotype diversity of *Listeria monocytogenes* strains associated with outbreaks of listeriosis in ruminants. *Journal of Clinical Microbiology*, 34(5), pp. 1086-1090. doi: 10.1128/jcm.34.5.1086-1090.1996

Wong, W.C., Pui, C.F., Tunung, R., Cheah, Y.K., Nakaguchi, Y., Nishibuchi, M. and Radu, S., 2012. Prevalence of *Listeria monocytogenes* in frozen burger patties in Malaysia. *International Food Research Journal*, 19(4), pp. 1751-1756.

World Health Organization., 2018. *Listeriosis*, [online] Available on: <https://www.who.int/news-room/fact-sheets/detail/listeriosis> [Accessed on 5 December 2021].

Wu, S., Wu, Q., Zhang, J., Chen, M., Yan, Z. and Hu, H., 2015. *Listeria monocytogenes* Prevalence and Characteristics in Retail Raw Foods in China. *PLoS ONE*, 10(8), e0136682. doi: 10.1371/journal.pone.0136682.

Wuenscher, M.D., Köhler, S., Bubert, A., Gerike, U. and Goebel, W., 1993. The *iap* gene of *Listeria monocytogenes* is essential for cell viability, and its gene product, p60, has bacteriolytic activity. *Journal of Bacteriology*, 175(11), pp. 3491-3501. doi: 10.1128/jb.175.11.3491-3501.1993

Yin, Y., Zhang, C., Dong, H., Niu, Z., Pan, Z., Huang, J. and Jiao, X., 2010. Protective immunity induced by a LLO-deficient *Listeria monocytogenes*. *Microbiology and Immunology*, 54(4), pp. 175-183. doi: 10.1111/j.1348-0421.2010.00211.x

Zhang, Y., Dong, S., Chen, H., Zhang, J., Zhang, Z., Yang, Y., Xu, Z., Zhan, L. and Mei, L., 2019. Prevalence, Genotypic Characteristics and Antibiotic Resistance of *Listeria monocytogenes* From Retail Foods in Bulk in Zhejiang Province, China. *Frontiers in Microbiology*, 10. doi: 10.3389/fmicb.2019.01710

Zhao, H., Chen, J., Fang, C., Xia, Y., Cheng, C., Jiang, L., and Fang, W., 2011. Deciphering the biodiversity of *Listeria monocytogenes* lineage III strains by polyphasic approaches. *The Journal of Microbiology*, 49(5), pp. 759-767.doi: 10.1007/s12275-011-1006-4

Zhao, T., Doyle, M.P. and Zhao, P., 2004. Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. *Applied and Environmental Microbiology*, 70, pp. 3996-4003. doi: 10.1128/AEM.70.7.3996-4003.2004

Zhao, T., Podtburg, T.C., Zhao, P., Chen, D., Baker, D.A., Cords, B. and Doyle, M.P., 2013. Reduction by competitive bacteria of *Listeria monocytogenes* in biofilms and *Listeria* bacteria in floor drains in a ready to eat poultry processing plant. *Journal of Food Protection*, 76, pp. 601-607. doi: 10.4315/0362-028X.JFP-12-323

APPENDIX

Details Information of Collected Samples

No.	Type	Category	Sample	Location	Temp
1	IFCS	Surface – Granite	Table top	Food Premise A	RT
2	IFCS	Surface – Granite	Table top	Food Premise A	RT
3	IFCS	Surface – Granite	Table top	Food Premise A	RT
4	IFCS	Surface – Granite	Table top	Food Premise A	RT
5	IFCS	Surface – Granite	Table top	Food Premise A	RT
6	FCS	Surface – PP	Cutting board	Food Premise B	RT
7	IFCS	Surface – PP	Table top	Food Premise B	RT
8	IFCS	Surface – PP	Food tray	Food Premise B	RT
9	FCS	Surface – SS	Surface for pre-cut fish	Hypermarket A	Cold
10	FCS	Surface – PP	Surface for whole fish	Hypermarket A	Cold
11	IFCS	Surface – PP	Freezer for frozen products	Hypermarket A	Freezer
12	IFCS	Surface – SS	Cold storage platform	Hypermarket A	Cold
13	IFCS	Surface – SS	Rack for dry products	Hypermarket A	RT
14	IFCS	Surface – SS	Weighing scale	Food Premise A	RT
15	FCS	Surface – Granite	Working table top	Food Premise A	RT
16	FCS	Surface – SS	Working table top	Food Premise A	RT
17	FCS	Surface – PP	Cutting board	Food Premise A	RT
18	FCS	Surface – PP	Bowl	Food Premise A	RT
19	FCS	Surface – SS	Knife	Food Premise A	RT
20	FCS	Surface – SS	Egg beater	Food Premise A	RT
21	IFCS	Surface – PP	Freezer bottom layer	Food Premise A	Freezer
22	IFCS	Surface – PP	Freezer upper layer	Food Premise A	Freezer
23	IFCS	Surface – PP	Freezer 3rd layer from the top	Food Premise A	Freezer
24	FCS	Surface – SS	Knife	Food Premise A	RT
25	FCS	Surface – PP	Brown colour bowl	Food Premise A	RT
26	FCS	Surface – PP	Cutting board	Food Premise A	RT
27	FCS	Surface – HDPE	Small plastic container	Food Premise A	RT
28	IFCS	Surface – HDPE	Food tray white with label	Food Premise A	RT
29	FCS	Surface – SS	Table top near centre part	Food Premise A	RT
30	FCS	Surface – SS	Table top near cooking stove	Food Premise A	RT
31	IFCS	Surface – PP	Refrigerator (fruit storage)	Food Premise A	RT
32	IFCS	Surface – PP	Refrigerator (1st layer storage)	Food Premise A	RT
33	IFCS	Surface – PP	Refrigerator (upper storage)	Food Premise A	RT
34	IFCS	Surface – Granite	Table top (food serving)	Food Premise C	RT
35	FCS	Surface – SS	Spoon	Food Premise C	RT
36	FCS	Surface – SS	Knife	Food Premise C	RT
37	FCS	Surface – SS	Shovel	Food Premise C	RT
38	FCS	Surface – SS	Working table	Food Premise C	RT
39	FCS	Surface – PP	Cutting board	Food Premise C	RT
40	FCS	Surface – PP	Cutting board	Food Premise C	RT
41	IFCS	Surface – SS	Table top	Food Premise D	RT
42	FCS	Surface – SS	Food stall corner bench	Food Premise D	RT

43	FCS	Surface – SS	Food stall bench	Food Premise D	RT
44	FCS	Surface – SS	Pan mee stall bench	Food Premise D	RT
45	IFCS	Surface – Wood	Table top	Food Premise E	RT
46	IFCS	Surface – SS	Table top of food stall	Food Premise D	RT
47	IFCS	Surface – Granite	Table top for chilli serving	Food Premise E	RT
48	FCS	Surface – SS	Serving plate	Food Premise C	RT
49	FCS	Surface – SS	Water dispenser	Food Premise A	RT
50	FCS	Surface – SS	Water dispenser	Food Premise A	RT
51	IFCS	Surface – Granite	Table top	Food Premise A	RT
52	IFCS	Surface – SS	Vegetarian stall bench	Food Premise A	RT
53	IFCS	Surface – SS	Vegetarian trolley	Food Premise A	RT
54	IFCS	Surface – SS	Long bench	Food Premise A	RT
55	IFCS	Surface – Granite	Table top	Food Premise A	RT
56	IFCS	Surface – Granite	Table top	Food Premise A	RT
57	IFCS	Surface – SS	Bench of food stall	Food Premise A	RT
58	IFCS	Surface – Granite	Table top	Food Premise A	RT
59	IFCS	Surface – Granite	Table top	Food Premise A	RT
60	IFCS	Surface – SS	Moving stall	Food Premise A	RT
61	IFCS	Surface – SS	Selling bench	Food Premise A	RT
62	FCS	Surface – PP	Ice box storage	Food Premise A	RT
63	FCS	Surface – SS	Water dispenser	Food Premise A	RT
64	FCS	Surface – SS	Water dispenser	Food Premise A	RT
65	IFCS	Surface – Granite	Table top	Food Premise A	RT
66	IFCS	Surface – Granite	Table top	Food Premise A	RT
67	FCS	Surface – SS	Water dispenser	Food Premise A	RT
68	FCS	Surface – SS	Water dispenser	Food Premise A	RT
69	RTE	Food – RTE	Fish ball	Night Market	RT
70	RTE	Food – RTE	Sausage	Night Market	RT
71	RTE	Food – RTE	Vege Sushi	Night Market	RT
72	RTE	Food – RTE	Egg mayo sushi	Night Market	RT
73	RTE	Food – RTE	Seafood sushi	Night Market	RT
74	RTE	Food – RTE	Tuna mix	Night Market	RT
75	RTE	Food – RTE	Rendang	Night Market	RT
76	RTE	Food – RTE	Chicken sandwich	Food Premise F	RT
77	RTE	Food – RTE	Egg sandwich	Food Premise F	RT
78	RTE	Food – RTE	Prawn/shrimp wantan	Hypermarket B	RT
79	RTE	Food – RTE	Cheese slice	Hypermarket B	RT
80	PF	Food – Meat	Chicken slice	Hypermarket B	RT
81	MPF	Food – Meat	Uncooked minced beef meat	Hypermarket B	RT
82	FCS	Surface – SS	Mo Pack table	Production Site A	RT
83	FCS	Surface – SS	Mo Pack table	Production Site A	RT
84	FCS	Surface – SS	Mo Pack conveyor belt	Production Site A	RT
85	FCS	Surface – SS	Mo Pack conveyor belt	Production Site A	RT
86	FCS	Surface – Teflon	Layer house belt	Production Site A	RT
87	FCS	Surface – Teflon	Layer house belt	Production Site A	RT
88	FCS	Surface – SS	Layer house	Production Site A	RT
89	FCS	Surface – SS	Layer house	Production Site A	RT
90	FCS	Surface – SS	KUHL table	Production Site A	RT
91	FCS	Surface – SS	KUHL table	Production Site A	RT
92	FCS	Surface – Rubber	KUHL table	Production Site A	RT
93	FCS	Surface – Rubber	KUHL table	Production Site A	RT
94	FCS	Surface – SS	OMINIA table	Production Site A	RT
95	FCS	Surface – PP	OMINIA blue transfer conveyor	Production Site A	RT

96	FCS	Surface – SS	OMINIA steel conveyor	Production Site A	RT
97	FCS	Surface – SS + PP	OMINIA orientation cup	Production Site A	RT
98	FCS	Surface – SS + PP	OMINIA orientation cup	Production Site A	RT
99	FCS	Surface – Food	Eggs before UV	Production Site A	RT
100	FCS	Surface – Food	Eggs before UV	Production Site A	RT
101	FCS	Surface – Food	Eggs after UV	Production Site A	RT
102	FCS	Surface – Food	Eggs after UV	Production Site A	RT
103	FCS	Surface – Food	Eggs after UV	Production Site A	RT
104	FCS	Surface – Rubber	OMINIA roller bar yellow	Production Site A	RT
105	FCS	Surface – Rubber	OMINIA roller bar yellow	Production Site A	RT
106	FCS	Surface – PP	OMINIA tray cup	Production Site A	RT
107	FCS	Surface – PP	OMINIA tray cup	Production Site A	RT
108	FCS	Surface – Paper	Paper egg tray	Production Site A	RT
109	FCS	Surface – Paper	Paper egg tray	Production Site A	RT
110	FCS	Surface – PP	OMINIA end table	Production Site A	RT
111	FCS	Surface – PP	OMINIA end table	Production Site A	RT
112	IFCS	Surface – PP	Pallet	Production Site A	RT
113	IFCS	Surface – SS	Packaging room table	Production Site A	RT
114	IFCS	Surface – SS	Packaging room table	Production Site A	RT
115	FCS	Surface – Rubber	PELBO breaker conveyor	Production Site A	RT
116	FCS	Surface – SS	PELBO breaker holder	Production Site A	RT
117	FCS	Surface – SS	PELBO breaker holder	Production Site A	RT
118	FCS	Surface – SS	PELBO table	Production Site A	RT
119	FCS	Surface – PP	Liquid eggs pail	Production Site A	RT
120	FCS	Surface – PP	Liquid eggs pail cover	Production Site A	RT
121	IFCS	Surface – PP	Pallet	Production Site A	RT
122	IFCS	Surface – SS	Pallet Jack Handle	Production Site A	RT
123	FCS	Surface – Food	Kampung eggs	Production Site A	RT
124	FCS	Surface – Food	Grade C eggs	Production Site A	RT
125	FCS	Surface – Food	Egg after UV	Production Site A	RT
126	FCS	Surface – Rubber	Belt UV	Production Site A	RT
127	FCS	Surface – Food	Egg after UV	Production Site A	RT
128	IFCS	Surface – SS	Table top for cold storage	Production Site B	Freezer
129	IFCS	Surface – PP	Racks	Production Site B	Freezer
130	IFCS	Surface – SS	Wall inside cold room	Production Site B	Freezer
131	IFCS	Surface – PP	Racks for food preparation	Production Site B	Freezer
132	IFCS	Surface – SS	Wall of freezer	Production Site B	Freezer
133	IFCS	Surface – SS	Rack stands for transfer food	Production Site B	RT
134	IFCS	Surface – SS	Stainless steel rack	Production Site B	RT
135	FCS	Surface – SS	Stainless steel bowl	Production Site B	RT
136	FCS	Surface – SS	Stainless steel sink	Production Site B	RT
137	FCS	Surface – SS	Stainless steel grinder/mixer	Production Site B	RT
138	FCS	Surface – SS	Working table	Production Site B	RT
139	FCS	Surface – SS	Working table	Production Site B	RT
140	FCS	Surface – Teflon	Conveyor belt for food transfer	Production Site B	RT
141	FCS	Surface – SS	Table top beside conveyor belt	Production Site B	RT
142	FCS	Surface – SS	Production line part	Production Site B	RT
143	FCS	Surface – SS	Apparatus for food transfer	Production Site B	RT
144	FCS	Surface – SS	Cooling machine	Production Site B	RT
145	FCS	Surface – SS	Frying machine	Production Site B	RT
146	IFCS	Surface – PP	Pallet for food storage	Production Site B	RT
147	FCS	Surface – PP	Cutting board	Production Site B	RT
148	FCS	Surface – PP	Food label for food preparation	Production Site B	RT

149	FCS	Surface – PP	Cutting board	Production Site B	RT
150	FCS	Surface – SS	Knife for cutting	Production Site B	RT
151	FCS	Surface – PP	Food tray (inner part)	Production Site B	RT
152	IFCS	Surface – PP	Food tray (outer part)	Production Site B	RT
153	FCS	Surface – SS	Multiple layer tray	Production Site B	RT
154	FCS	Surface – SS	Working table top	Production Site B	RT
155	FCS	Surface – Teflon	Conveyor belt	Production Site B	RT
156	FCS	Surface – SS	Weighing scale	Production Site B	RT
157	IFCS	Surface – PP	Pallet for food storage	Production Site B	Cold
158	IFCS	Surface – PP	Racks for food storage	Production Site B	Cold
159	IFCS	Surface – SS	Wall of cold room	Production Site B	Cold
160	IFCS	Surface – PP	Racks for cold room	Production Site B	Freeze
161	RM	Food – Meat	Egg	Production Site A	RT
162	RM	Food – Meat	Egg	Production Site A	RT
163	RM	Food – Vege	Corn	Wet market	RT
164	RM	Food – Vege	Bean sprouts	Wet market	Cold
165	RTE	Food – RTE	Fried wantan	Wet market	RT
166	RTE	Food – RTE	Fried food	Wet market	RT
167	RM	Food – Vege	Bean sprouts	Wet market	Cold
168	RTE	Food – RTE	Fried bean curd	Wet market	RT
169	RM	Food – Vege	Fresh bean curd	Wet market	RT
170	RTE	Food – RTE	Fish cake	Wet market	RT
171	RTE	Food – RTE	Fish cake in round shape	Wet market	RT
172	RTE	Food – RTE	Fried food	Wet market	RT
173	RTE	Food – RTE	Fried bean curd stick	Wet market	RT
174	MPF	Food – Meat	Minced chicken meat	Wet market	RT
175	MPF	Food – Meat	Chicken breast in pieces	Wet market	RT
176	RTE	Food – Vege	Cherry tomato	Hypermarket A	RT
177	RTE	Food – Vege	Cucumber	Hypermarket A	RT
178	RM	Food – Vege	Sweet potato	Hypermarket A	RT
179	RM	Food – Vege	Bok Choi	Hypermarket A	Cold
180	RM	Food – Vege	Peanuts (Raw, not roasted)	Hypermarket A	RT
181	PF	Food – Meat	Chicken sausage	Hypermarket A	Freezer
182	RTE	Food – RTE	Starfruits (Pre-cut)	Hypermarket A	Cold
183	RTE	Food – RTE	Watermelon (Pre-cut)	Hypermarket A	Cold
184	RTE	Food – RTE	Watermelon (Pre-cut)	Hypermarket A	Cold
185	RTE	Food – RTE	Pineapple (Pre-cut)	Hypermarket A	Cold
186	MPF	Food – Vege	Green pepper (Pre-cut)	Hypermarket A	Cold
187	MPF	Food – Vege	Carrot (Pre-cut)	Hypermarket A	Cold
188	MPF	Food – Vege	White cabbage (Pre-cut)	Hypermarket A	Cold
189	MPF	Food – Vege	White cauliflower (Pre-cut)	Hypermarket A	Cold
190	MPF	Food – Vege	Broccoli (Pre-cut)	Hypermarket A	Cold
191	RM	Food – Vege	Bean sprouts	Wet market	Cold
192	RM	Food – Vege	Bean sprouts	Wet market	Cold
193	RM	Food – Vege	Bok Choi	Wet market	RT
194	RTE	Food – Vege	Mint leaves	Wet market	RT
195	RM	Food – Vege	Okra	Wet market	RT
196	RM	Food – Vege	Cucumber	Wet market	RT
197	RM	Food – Vege	Cabbage	Wet market	RT
198	RTE	Food – Vege	Mint leaves	Wet market	RT
199	RM	Food – Vege	Baby sweet corn	Wet market	RT
200	MPF	Food – Vege	Sweet corn	Wet market	RT
201	RM	Food – Vege	Sweet potato	Wet market	RT

202	RM	Food – Vege	White turnip	Wet market	RT
203	RTE	Food – RTE	Fried fish cake	Wet market	RT
204	MPF	Food – Meat	Fish paste	Wet market	RT
205	MPF	Food – Meat	Chicken breast in pieces	Wet market	RT
206	RTE	Food – RTE	Turnover pancake	Night Market	RT
207	RTE	Food – RTE	Steamed egg sponge cake	Night Market	RT
208	RTE	Food – RTE	Butter cake	Night Market	RT
209	RTE	Food – RTE	Swiss roll cake	Night Market	RT
210	RTE	Food – RTE	Chicken ham sandwich	Night Market	RT
211	RTE	Food – RTE	Fried sweet potato	Night Market	RT
212	RTE	Food – RTE	Fried bread with sausage	Night Market	RT
213	RTE	Food – RTE	Fried bread with sardine	Night Market	RT
214	RTE	Food – RTE	Sardine sandwich (non-fried)	Night Market	RT
215	RTE	Food – RTE	Egg sandwich (non-fried)	Night Market	RT
216	RTE	Food – RTE	Popiah	Night Market	RT
217	RTE	Food – RTE	Crispy prawn fritters	Night Market	RT
218	RTE	Food – RTE	Hard-boiled egg	Night Market	RT
219	RTE	Food – RTE	Rice	Night Market	RT
220	RTE	Food – RTE	Fried anchovies	Night Market	RT
221	MPF	Food – Vege	Pre-cut mushroom	Hypermarket B	Cold
222	MPF	Food – Vege	Pre-cut mushroom	Hypermarket B	Cold
223	RTE	Food – RTE	Fried onion	Hypermarket B	Cold
224	RM	Food – Vege	Bean sprouts	Hypermarket B	Cold
225	RM	Food – Vege	Broccoli	Hypermarket B	Cold
226	RM	Food – Vege	White cabbage	Hypermarket B	Cold
227	RTE	Food – Vege	Cucumber	Hypermarket B	RT
228	RTE	Food – RTE	Garlic bread	Hypermarket B	RT
229	RM	Food – Vege	Chinese chives	Hypermarket B	Cold
230	RM	Food – Vege	Enoki mushroom	Hypermarket B	Cold
231	RTE	Food – Vege	Green coral lettuce	Hypermarket B	Cold
232	MPF	Food – Meat	Prawn (processed)	Hypermarket B	Cold
233	MPF	Food – Meat	Chicken fillet	Hypermarket B	Cold
234	RTE	Food – RTE	Chicken roll slice	Hypermarket B	Cold
235	PF	Food – Meat	Chicken sausage	Hypermarket B	Cold
236	RTE	Food – RTE	Yellow noodles	Hypermarket C	RT
237	RTE	Food – RTE	Dried chilli	Hypermarket C	RT
238	MPF	Food – Vege	Baby sweet corn	Hypermarket C	Cold
239	MPF	Food – Meat	Prawn (processed)	Hypermarket C	Cold
240	RM	Food – Vege	Purple cabbage	Hypermarket C	RT
241	RTE	Food – Vege	Cucumber	Hypermarket C	RT
242	RM	Food – Vege	Pumpkin	Hypermarket C	RT
243	RM	Food – Vege	Tomato	Hypermarket C	RT
244	RM	Food – Vege	Potato	Hypermarket C	RT
245	RM	Food – Vege	Sweet potato	Hypermarket C	RT
246	MPF	Food – Vege	Bean curd	Hypermarket C	Cold
247	MPF	Food – Meat	Chicken fillet	Hypermarket C	Cold
248	RTE	Food – RTE	Chocolate Ice-cream	Hypermarket C	Freezer
249	RTE	Food – RTE	Vanilla Ice-cream	Hypermarket C	Freezer
250	RTE	Food – RTE	Blueberry Ice-cream	Hypermarket C	Freezer
251	RTE	Food – RTE	Pineapple biscuit	Hypermarket C	RT
252	RTE	Food – RTE	Chocolate cup cake	Food Premise F	RT
253	RTE	Food – RTE	Marble cup cake	Food Premise F	RT
254	RTE	Food – RTE	Blueberry cup cake	Food Premise F	RT

255	RTE	Food – RTE	Sausage puff	Hypermarket C	RT
256	RTE	Food – RTE	Curry chicken puff	Hypermarket C	RT
257	RTE	Food – RTE	Sausage	Food Premise F	RT
258	PF	Food – Meat	Smoked duck	Wet market	RT
259	PF	Food – Meat	Smoked duck	Wet market	RT
260	RM	Food – Vege	Potato	Hypermarket C	RT
261	RTE	Food – RTE	Instant coffee powder	Food Premise F	RT
262	MPF	Food – Vege	Pre-cut onion	Hypermarket D	Cold
263	MPF	Food – Vege	Pre-cut peppers	Hypermarket D	Cold
264	MPF	Food – Vege	Pre-cut ginger slices	Hypermarket D	Cold
265	MPF	Food – Vege	Pre-cut lemongrass	Hypermarket D	Cold
266	MPF	Food – Vege	Pre-cut chicken wings	Hypermarket D	Cold
267	RTE	Food – RTE	Potato salad	Hypermarket D	Cold
268	RTE	Food – RTE	Chicken ham sandwich	Hypermarket D	Cold
269	RTE	Food – RTE	Tuna sandwich with oat bread	Hypermarket D	Cold
270	RTE	Food – RTE	Fried egg	Hypermarket D	RT
271	RTE	Food – RTE	Rice	Hypermarket D	RT
272	RTE	Food – RTE	Sambal	Hypermarket D	RT
273	MPF	Food – Vege	Pre-cut fried bean curd puff	Hypermarket D	Cold
274	MPF	Food – Vege	Pre-cut red pepper	Hypermarket D	Cold
275	MPF	Food – Vege	Pre-cut carrot	Hypermarket D	Cold
276	MPF	Food – Vege	Mushroom	Hypermarket D	Cold
277	MPF	Food – Vege	Pre-cut broccoli	Hypermarket D	Cold
278	MPF	Food – Vege	Pre-cut cauliflower	Hypermarket D	Cold
279	RTE	Food – RTE	Cheese	Hypermarket D	Cold
280	MPF	Food – Meat	Salmon	Hypermarket D	Cold
281	RTE	Food – RTE	Cream puff	Food Premise F	Cold
282	RTE	Food – RTE	Marble butter cake	Food Premise F	RT
283	RTE	Food – RTE	Butter cake	Food Premise F	RT
284	RTE	Food – RTE	Sponge cake	Food Premise F	RT
285	RTE	Food – RTE	Cappuccino cake	Night Market	RT
286	RTE	Food – RTE	Mascarpone cake	Night Market	RT
287	RTE	Food – RTE	Oreo cheese	Night Market	RT
288	RTE	Food – RTE	Donut	Night Market	RT
289	RTE	Food – RTE	Fried chicken	Night Market	RT
290	RTE	Food – RTE	Satay chicken	Night Market	RT
291	RTE	Food – RTE	Cucumber sauce	Night Market	RT
292	RTE	Food – RTE	Pre-cut guava	Hypermarket D	Cold
293	RTE	Food – RTE	Pre-cut cantaloupe	Hypermarket D	Cold
294	RTE	Food – RTE	Pre-cut watermelon	Hypermarket D	Cold
295	MPF	Food – RTE	Pre-cut pumpkin	Hypermarket D	RT
296	RTE	Food – RTE	Grape	Hypermarket D	Cold
297	RTE	Food – RTE	Kiwi	Hypermarket D	Cold
298	RTE	Food – RTE	Berries	Hypermarket D	Cold
299	RTE	Food – RTE	Pineapple	Hypermarket D	Cold
300	RTE	Food – RTE	Cantaloupe	Hypermarket D	Cold
301	RTE	Food – RTE	Salmon sushi roll	Hypermarket D	Cold
302	RTE	Food – RTE	Cucumber sushi roll	Hypermarket D	Cold
303	RTE	Food – RTE	Seafood roll	Hypermarket D	Cold
304	RTE	Food – RTE	Salmon slice	Hypermarket D	Cold
305	RTE	Food – RTE	Pepperoni slice	Hypermarket D	Cold
306	RTE	Food – RTE	Salmon skin	Hypermarket D	Cold
307	MPF	Food – Meat	Shi Ban Fish	Wet Market	RT

308	RM	Food – Meat	Chicken Wing	Wet Market	RT
309	RM	Food – Meat	Chicken Wing	Wet Market	RT
310	RM	Food – Meat	Chicken Foot	Wet Market	RT
311	MPF	Food – Meat	Fish	Wet Market	RT
312	RM	Food – Meat	Prawn	Wet Market	RT
313	RM	Food – Meat	Chicken gizzard	Wet Market	RT
314	RM	Food – Meat	Chicken liver	Wet Market	RT
315	PF	Food – Meat	Beef patties	Hypermarket B	Freezer
316	RM	Food – Meat	Chicken gizzard	Wet Market	RT
317	RM	Food – Meat	Chicken liver	Wet Market	RT
318	IFCS	Surface – Cloth	Cleaning cloth	Food Premise A	RT
319	IFCS	Surface – Cloth	Cleaning cloth	Food Premise B	RT
320	IFCS	Surface – Cloth	Cleaning cloth	Food Premise C	RT
321	IFCS	Surface – Cloth	Cleaning cloth	Food Premise D	RT
322	IFCS	Surface – Cloth	Cleaning cloth	Food Premise E	RT

PUBLICATION AND SYMPOSIUM PARTICIPATION

The manuscript generated based on the findings of the first objective of this study has been accepted for publication in the Food Research. In addition, some parts of this study's data were presented in four conferences in the form of oral or poster presentations that held in 2018 to 2021: a) ASEAN Emerging Researchers Conference on 3rd of December 2018; b) 11th MIFT National Food Science and Technology Competition on 6th and 7th of April 2019; 2nd Biennial Medical and Health Sciences Conference on 4th to 6th of July 2019; and d) 12th MIFT National Food Science and Technology Competition on 4th and 5th of August 2021.

Conference Proceeding:

Chen, S.N., Yap, M.L., Kuan, C.H., Kuan, C.S. and Saw, S.H., 2019. Optimization of Polymerase Chain Reaction (PCR) Conditions for Detection of *Listeria monocytogenes* from Food Samples. *Malaysian Journal of Pathology*, 41(3), pp. 447. http://www.mjpath.org.my/2019/v41n3/Abstracts-BMHSC.pdf

Publication reference:

Chen, S.N., Yap, M.L., Kuan, C.H., Son, R. and Saw, S.H., 2021. Could food or food contact surfaces be the favourable hideouts for *Listeria monocytogenes* in Perak, Malaysia? *Food Research*, 5(3), pp. 174-182, doi:10.26656/fr.2017.5(3).596