MICROBIAL RISKS ASSOCIATED WITH READY-TO-EAT FOODS

LEE HUI KEY

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2018

MICROBIAL RISKS ASSOCIATED WITH READY-TO-EAT FOODS

LEE HUI KEY

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR
2018

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: (I.C/Passport No:)

Registration/Matric No:

Name of Degree:

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

Field of Study:

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

MICROBIAL RISKS ASSOCIATED WITH READY-TO-EAT FOODS

ABSTRACT

Numerous reported food poisoning incidents due to microbiological contamination in ready-to-eat (RTE) foods in Malaysia were related to academic institutions. Therefore, this study aimed to (i) examine the microbiological quality of RTE foods, food contact surfaces (FCS), table cleaning cloths (TCC), and food handlers' hands in the food premises of a public university; (ii) determine the antimicrobial profile, virulence profile and genetic relatedness of bacteria isolated; and (iii) assess the food handlers' knowledge, attitude and practices (KAP) on food safety. A total of 150 RTE foods, 59 FCS, 34 TCC, and 85 food handlers' hands swab samples were determined for aerobic colony count (ACC), coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Vibrio cholerae,* and *Vibrio parahaemolyticus*. The bacteria isolated were then characterised by antimicrobial susceptibility testing, virulotyping and genotyping. Sixty-seven (n = 67) food handlers were recruited on the voluntary basis to study the KAP on food safety. Fifty percent (75/150) of the RTE foods harboured an unsatisfactory level of ACC, while 24% (36/150) carried >4 Log CFU/g of coliforms. *Salmonella* spp. was detected in 33% (50/150) of the RTE foods, 37% (22/59) of the FCS, 62% (21/150) of the TCC and 48% (41/85) of the food handlers' hands. All RTE foods, 90% of FCS (53/59) and 82% of TCC (28/34) sampled had satisfactory level of *Staph. aureus* count. Only 5% (3/59), 12% (4/34), and 35% (30/85) of the FCS, TCC and food handlers' hand, respectively had a satisfactory level of ACC, respectively. The food handlers had moderate food safety knowledge (61.8%), positive attitudes and practices. The education level, working experience and the food safety training course significantly improved the knowledge and attitude of the food handlers (p <0.05). It was noticed that the knowledge on proper food handling practices was not translated into real practices, which results in the poor microbiological quality of the food handlers' hands, FCS, and the RTE foods prepared.

In this study, 130 isolates of *E. coli*, 81 isolates of *Staph. aureus* and 26 isolates of *V. cholerae* were recovered from RTE foods, FCS, TCC and food handler's hands. All *E. coli* isolated were non-virulent, but one-third was multidrug resistant. About 97.5% of the *Staph. aureus* and 88.5% of the *V. cholerae* strains carried ≥ 1 virulence gene. Cross contamination could have happened among TCC, FCS, and food handlers as 100% similarity among the strains isolated from these samples was observed. This study revealed the high unsatisfactory level of aerobic colony count and *Salmonella* spp. contamination. The food handlers had poor hand sanitation despite perceiving adequate knowledge, good attitudes and self-reported practices. Therefore, the current safe food handling course needs to be reviewed, and the authority should have a closer monitoring to ensure the food handlers practice proper food handling.

Keywords: Food safety; microbiological risks, ready-to-eat foods

RISIKO MIKROBIAL YANG BERKAITAN DENGAN MAKANAN YANG SEDIA DIMAKAN

ABSTRAK

Kebanyakan peristiwa keracunan makanan di Malaysia yang dilaporkan adalah disebabkan oleh pencemaran mikroorganisma yang sering berhubung kait dengan institusi-institusi pengajian. Oleh itu, penyelidikan ini bertujuan untuk (i) mengkaji kualiti mikrobiologi makanan yang sedia dimakan (RTE), permukaan yang disentuhi makanan (FCS), kain lap meja (TCC) dan tangan pengendali makanan di suatu universiti awam; (ii) menentukan profil antimikrob, profil kevirulenan, dan kaitan genetik di antara semua bakteria yang dipencilkan, dan (iii) menilai pengetahuan, sikap dan amalan-yang-dilaporkan (KAP) terhadap keselamatan makanan dalam kalangan pengendali makanan. Terdapat 150 makanan RTE, 59 FCS, 34 TCC dan 85 pengendali makanan telah ditentukan kiraan koloni aerobic (ACC), koliform, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Vibrio cholerae* dan *Vibrio parahaemolyticus*. Bakteria yang dipencilkan telah dicirikan dengan kerentanan antimikroorganisma, virulotaip dan genotaipnya. Enam puluh tujuh orang pengendali makanan telah direkrut dengan sukarela untuk mengkaji KAP keselamatan makanan.Lima puluh peratus (75/150) makanan RTE mengandungi tahap ACC yang tidak memuaskan, manakala 24% mengandungi tahap koliform yang melebihi 4 log CFU/g. *Salmonella* spp. telah dikesan pada 33% (50/150) makanan RTE, 37% (22/59) FCS, 62% (21/150) TCC, dan 48% (41/48) pada tangan pengendali makanan. Semua makanan RTE, 90% FCS (53/59) dan 82% TCC (28/34) yang disampelkan mempunyai tahap kiraan koloni *Staph. aureus* yang memuaskan. Hanya 5% (3/59) FCS, 12% (4/34) TCC, dan 35% (30/85) tangan pengendali makanan yang mengandungi tahap ACC yang memuaskan. Pengendali makanan mempunyai pengetahuan yang sederhana (61.8%) terhadap keselamatan makanan, sikap dan kelakuan yang dilaporkan yang positif. Tahap pendidikan, pengalaman bekerja dan kursus pengendalian makanan menambah baik pengetahuan dan sikap pengendali makanan

terhadap keselamatan makanan secara bererti (p <0.05). Pengetahuan tentang pengendalian makanan yang wajar tidak dipraktikan, oleh itu mengakibatkan kualiti microbiologi pada tangan pengendalian makanan, FCS dan juga makanan yang disediakan kurang memuaskan. Dalam kajian ini, 130 *E. coli*, 81 *Staph. aureus* and 26 *V. cholerae* telah dipencilkan dari makanan RTE, FCS, TCC dan tangan pengendali makanan. Semua *E. coli* tidak mengandugi gen virulen tetapi satu-pertiga merupakan strain rintang pelbagai dadah. Lebih kurang 97.5% strain *Staph. aureus* dan 88.5% strain *V. cholerae* mengandungi sekurang-kurangnya satu gen virulen. Pencemaran silang mungkin berlaku di antara TCC, FCS dan tangan pengendali makanan kerana terdapat pencilan-pencilan dari sampel-sampel ini mengandungi 100% persamaan dalam genetik. Kajian ini menyatakan pencemaran yang tidak memuaskan terhadap ACC dan *Salmonella* spp.. Pengendali makanan mengamalkan kebersihan tangan yang kurang memuaskan, walaupun memperolehi pengetahuan terhadap keselamatan makanan, sikap dan amalan-yang-dilaporkan yang positif. Oleh itu, kursus pengendalian makanan semasa perlu dikaji semula dan pihak berkuasa juga perlu mengadakan pengawasan yang lebih kerap untuk memastikan pengendali makanan mempraktikan pengendalian makanan yang sewajarnya.

ACKNOWLEDGEMENTS

Above all, I would like to express my heartfelt gratitude to my supervisors, Prof. Dr Thong Kwai Lin and Dr Chai Lay Ching who had given me so much guidance and patience with my research. Without them, my dissertation would not have been completed.

I would also like to thank the University of Malaya Research Grant (RP003A-13BIO and RP003C-13BIO) for supporting my study. My study was also supported by MyMaster after the UMRG grant has ended.

Besides that, I am grateful that I have a group of supporting lab mates, especially Soo Tein, Wing Sze, Tony, Xiu Pei, Shiang Chiet, Hannah and others who had given me so much inspirations and motivations. Last but not least, the GOD has been good to me that I have a good mother who has been very supportive throughout my life.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

Symbols

℃	:	Degree Celcius
µL	:	Microlitre
µM	:	Micromole
%	:	Percentage

Abbreviations

ATCC	:	American Type Culture Collection
bp	:	Base pair
BSA	:	Bovine serum albumin
cAMP	:	Cyclic adenosine 5-monophosphate
CCA	:	Codex Commission Alimentarius
CDC	:	Centre of Disease Control and Prevention
CFU	:	Colony forming unit
DAEC	:	Diffusely adherent *E. coli*
ddH_2O	:	Double distilled water
DNA	:	Deoxyribonucleic Acid
dNTP	:	Deoxynucleotide triphosphate
EAEC	:	Enteroaggregative *E. coli*
EC	:	*Escherichia coli*
EDTA	:	Ethylenediaminetetraacetic acid
egc	:	Enterotoxin gene cluster
EIEC	:	Enteroinvasive *E. coli*
EPEC	:	Enteropathogenic *E. coli*
et al.	:	Et alia

ETEC	:	Enterotoxigenic *E. coli*
FAO	:	Food and Agriculture Organization of the United Nation
FCS	:	Food contact surfaces
FDA	:	Food and Drug Administration
FSIS	:	United States Department of Agriculture Food Safety Inspection and Service
g	:	Gram
h	:	Hour
HACCP	:	Hazard Analysis Critical Control Point
HGT	:	Horizontal gene transfer
hlg	:	Gamma-haemolysin
hly	:	Haemolysin
KAP	:	Knowledge, attitude and practices
kb	:	Kilo base pair
L	:	Litre
LBA	:	Luria-Bertani agar
LBB	:	Luria-Bertani broth
log	:	Logarithm
M	:	Molar
MDR	:	Multidrug resistant
MDR	:	Multidrug-resistant
mg	:	Miligram
$MgCl_2$	:	Magnesium chloride
min	:	Minute
mL	:	Mililitre
MOH	:	Ministry of Health

mPCR	:	Multiplex polymerase chain reaction
MPN	:	Most probable number
MPN-PCR	:	Most probable number-polymerase chain reaction
MRA	:	Microbial risk assessment
na	:	Not available
NaCl	:	Sodium chloride
ND	:	Not detected
nk	:	Not known
NTS	:	Non-typhoidal salmonellosis
ompC	:	Outer membrane protein C
p	:	*p* value
PCR	:	Polymerase chain reaction
PFGE	:	Pulsed-field gel electrophoresis
pvl	:	Panton-Valentine Leukocidine
REP	:	Repetitive extragenic palindromic
rpm	:	Revolutions per minute
r_s	:	Spearman Rho
RTE	:	Ready-to-eat
SA	:	*Staphylococcus aureus*
SaPI	:	Staph. aureus Pathogenic Island
sea	:	Staphylococcal enterotoxin A
seb	:	Staphylococcal enterotoxin B
sec	:	Second
sec	:	Staphylococcal enterotoxin C
sed	:	Staphylococcal enterotoxin D
see	:	Staphylococcal enterotoxin E

seg	:	Staphylococcal enterotoxin G
sei	:	Staphylococcal enterotoxin I
sej	:	Staphylococcal enterotoxin J
SFP	:	Staphylococcal food poisoning
seh	:	Staphylococcal enterotoxin H
spp.	:	Species
STEC	:	Shigatoxin producing E. coli
Taq	:	*Thermus aquaticus*
TBE	:	Tris-borate EDTA
TCC	:	Table cleaning cloths
TE	:	Tris-EDTA
tox	:	Toxin gene
TSA	:	Tryptic soy agar
TSB	:	Tryptic soy broth
U	:	Unit
UPEC	:	Uropathogenic *E. coli*
UPGMA	:	Upweighted pair group method with arithmetic averages
UV	:	Ultraviolet
V	:	Volt
VC	:	*Vibrio cholerae*
VP	:	*Vibrio parahaemolyticus*

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Food safety is a major concern of the United Nation (UN) and World Health Organization (WHO). Food safety was set as the major agenda in 2015 (WHO, 2015b). It was noted that almost 2 million people died of foodborne illnesses yearly (WHO, 2015b). In Malaysia, foodborne illnesses have increased steadily in the recent years. The incidents of food poisoning reported in Malaysia were always associated with the academic institutions (MOH, 2014b).

In Malaysia, ready-to-eat (RTE) foods are easily available at affordable prices. Locals frequently have their meals at the workplace, street hawker stalls or restaurants due to inflexible working and school hours. However, the food safety of the RTE and food hygiene of these food premises are uncertain.

In addition, the cooking environment is a salient factor to ensure food safety and hygiene. A clean cooking environment could reduce the cross contamination and recontamination event. Food contact surfaces (FCSs) and table cleaning cloths (TCCs) are amongst the possible vehicles of transmission for food pathogens (Mattick et al., 2003; Perez-Rodriguez et al., 2008). Hence, the microbiological quality of these items is an important risk to be notified.

Improper food handling was identified as the main contributing factor for foodborne illnesses in Malaysia (MOH, 2007). Usually, the foods were prepared early in the morning and kept at room temperature. The improper storage temperature, long incubation time and unhygienic practices promote the bacterial growth and cross contamination event (Soon et al., 2011). Food handlers are involved in almost all the steps in food preparation. Thus, they play a critical role in ensuring food safety.

Knowledge, attitude, and practices (KAP) of the food handlers are the three main components that directly affect food safety. To improve the food safety in Malaysia, Ministry of Health (MOH) has made the safe food handling course as the prerequisite for

people to work in food service area. However, the effectiveness of this safe food handling course needs to be assessed and evaluated from time to time.

This study was conducted to answer a few research questions:

1) What is the microbiological quality of the ready-to-eat foods sold and the environmental factors in food premises of the selected public university?
2) What is the phenotypic and genotypic characteristics of the bacteria isolated from the ready-to-eat foods, food contact surfaces, table cleaning cloths and food handlers' hands?
3) How good is the food safety knowledge, attitudes and practices of the food handlers in the selected public university?

The objectives of this study were to

1) examine the microbiological quality of the ready-to-eat foods, food contact surfaces, table cleaning cloths, and food handlers' hands;
2) determine the antimicrobial susceptibility profile, virulence profile and genetic diversity of the *Escherichia coli*, *Staphylococcus aureus,* and *Vibrio cholerae and Vibrio parahaemolyticus* isolated from the ready-to-eat foods, food contact surfaces, table cleaning cloths, and food handlers' hands;
3) assess the food safety knowledge of the food handlers on food safety.

CHAPTER 2: LITERATURE REVIEW

2.1 Food Safety

Safe food is one of the core needs for human survival. In this era of globalisation, food safety can be a huge challenge due to the increase in human population, the commercialisation of food ingredients, the emergence of new foodborne diseases or the national food safety system.

The tremendous increase in human population is attributed to the problem in supplying sufficient food. At the same time, consumers are demanding for a variety of foods (Fukuda, 2015). Many food ingredients are imported from many countries. Therefore, the food contaminants can travel to a new place via this channel.

The World Health Organization (WHO) has been working closely with the United Nations Food and Agriculture Organization (FAO/WHO, 1983) to promote food safety. They act as the international referral which timely provides information and develops the guidelines to cope the food safety emergencies (WHO, 2015a). WHO and FAO also collaborate with Codex Alimentarius to develop Hazard Analysis and Critical Control Point (HACCP).

HACCP is a scientific-based system which identifies and controls specific hazards in ensuring food safety (Codex Alimentarius Commission, 2001b). It was initially implemented in the production line (Panisello & Quantick, 2001). HACCP was found to have a high impact on the regulations of food safety (Cormier et al., 2007). Therefore, it was implemented by the government sectors (Unnevehr & Jensen, 1999). The implementation of HACCP in food manufacturing was more likely than food service due to the difficulty in monitoring and the involvement of complicated food preparations (Mortlock et al., 2005).

In parallel with the implementation of HACCP, Ministry of Health (MOH) has suggested the Malaysian Certification Scheme for HACCP (FAO/WHO, 1983). On the

other hand, our government also mandated Food Handlers' Training Programme or Sijil Latihan Pengendalian Makanan (SLPM) since 1996 to promote safe food handling (Soon, Singh, & Baines, 2011). However, it requires full cooperation from all sectors, especially the management and the employees.

2.2 Foodborne disease

Foodborne disease can be caused by the ingestion of foods contaminated with microorganisms, chemicals, toxins or heavy metals. The occurrence of two or more same illness was considered as foodborne disease outbreaks (CDC, 2013c). Foodborne poisoning is always associated with gastroenteritis (diarrhoea and vomiting) or fever. The infants, pregnant lady, senior adults and immunocompromised patients are considered as a high-risk group as they will be more susceptible to foodborne diseases.

WHO (2015a) estimated almost 1 in 10 people fall sick yearly due to foodborne diseases while 420 000 cases were fatal. Children aged under five were accounted for one-third of the foodborne disease deaths (WHO, 2015a). South East Asia and African countries had higher foodborne burden than other continents (WHO, 2015a). Norovirus, *E. coli*, *Campylobacter* and non-typhoidal *Salmonella,* were the top causative agents for world foodborne diseases (WHO, 2015a).

In the United States of America (USA), 818 outbreaks were reported which caused 13360 illnesses and 16 deaths in 2012 (CDC, 2013c). Although Norovirus was listed as the most common foodborne pathogens in the outbreaks reported, but *Salmonella* accounted for a higher percentage of foodborne deaths (CDC, 2014b). CDC also revealed that restaurant foods accounted for 60% of the outbreaks while the caterers and home cooks caused 14% and 12% of the outbreaks, respectively (CDC, 2015a).

The accuracy of the statistics and epidemiology of foodborne illnesses depend on the efficiency of the reporting system. The USA has been developed a web-based platform

for the surveillance of foodborne disease since 2009. It is known as National Outbreak Reporting System (NORS): http://www.cdc.gov/nors/. These reported data are available online to the public at Foodborne Outbreak Online Database (FOOD Tool): http://wwwn.cdc.gov/foodborneoutbreaks/.

2.2.1 Foodborne diseases in Malaysia

Foodborne diseases are not rare in our country. There were 17 059 incidents of foodborne and waterborne diseases reported in 2015 (MOH, 2015). However, the incidence rate may be underestimated. Many sporadic cases might be underreported as the patients did not seek medical consultation. Our foodborne disease surveillance data was collected based on physician reports and the outbreak investigation (Soon et al., 2011). Therefore, the actual incidence rate is an unknown.

In Malaysia, there are five categories of foodborne diseases: cholera, dysentery, food poisoning, viral Hepatitis A and typhoid fever. Other foodborne diseases like shigellosis, listeriosis, and non-typhoidal salmonellosis are categorised as food poisoning. From 2013 to 2015, there was an increase in foodborne and waterborne diseases, particularly food poisoning (MOH, 2013, 2014a, 2015). This scenario could be due to the increase in non-typhoidal salmonellosis.

The main contributory factor to foodborne diseases in Malaysia is unhygienic food handling which causes more than 50% food poisoning cases (MOH, 2007). Approximately 43% of the foodborne diseases are associated with academic institutions (MOH, 2014b) which include boarding schools and school canteens. Foods were prepared in the morning and served during the recess time (around 11.00 am) and after school in the afternoon. The improper storage temperature until the food is served could increase the risk of food contamination (Soon et al, 2011). The high rates of incidence of foodborne

disease in academic institution indicate the need to improve food safety of RTE foods in food premises of the academic institutions.

2.3 Common etiologic agents

2.3.1 *Salmonella* spp.

Salmonella is one of the members of Enterobacteriaceae, which consists of only two species: *Salmonella bongori* and *Salmonella enterica* (*S. enterica*). *S. enterica* is then further subdivided into more than 2500 serotypes. These serotypes are identified by its antigens structures: flagellar "H," somatic "O" and polysaccharide "Vi."

All members of Salmonellae are able to infect humans. It can cause four different manifestations in human or salmonellosis: bacteraemia, gastroenteritis, enteric fever and asymptomatic carrier state (Ryan & Ray, 2004). It is transmitted via faecal-oral route. Patients develop fever, diarrhoea and abdominal cramps within 12- 72 hours after infection (CDC, 2015c). Often, the patients are able to recover completely from infection without the antibiotic treatment, unless the patients develop severe illnesses, or they are in the high-risk group (young children, pregnant women, senior citizens and immunocompromised patients) (CDC, 2015b).

S. enterica has been identified as the top etiologic agents of the foodborne disease. Non-typhoidal salmonellosis (NTS) is caused by non-typhoidal *Salmonella* such as *S. enterica* subsp. *enterica* serovar Typhimurium and *S.enterica* subsp. *enterica* serovar Enteritidis, the two most common serotypes worldwide, especially in African continents.

A study on the global burden of NTS by Majowicz *et al.* (2010) estimated 2.5 million cases and 400 deaths yearly in African nations. Although many studies have shown the associations between the incidence of NTS and home-cooked food (Taulo et al., 2008), market food (Hang'ombe et al., 1999) and fish, but further subtyping was not done to investigate the outbreaks (Feasey, Dougan, Kingsley, Heyderman, & Gordon, 2012).

On the contrary, typhoidal salmonellosis has frequently been identified in Asia (Ochiai et al., 2008). Unlike NTS, typhoidal salmonellosis is entirely restricted to the human host and could cause invasive disease (Feasey et al., 2012). Typhoidal salmonellosis is the dominant communicable disease in Malaysia (MOH, 2007). Therefore, all food handlers are compulsory to be vaccinated (Typhim Vi) against typhoid fever. Food handlers who are not vaccinated are not eligible to work in food service and food production areas. However, this vaccine needs a booster after every two years (CDC, 2013d). Typhoidal salmonellosis is associated with sanitation, and it could be eliminated by improving sanitation (Feasey et al., 2012).

Antimicrobial therapy will be given to a patient once he/she develops serious illness. Chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole are drugs of choice to treat salmonellosis (CDC, 2013d). Due to the emergence of *Salmonella* resistant to these drugs, fluoroquinolone and ceftriaxone are used as the alternative drug to treat multidrug-resistant (MDR) *Salmonella* (Asperilla et al., 1987). In the year 1999, several groups of researchers discovered the emergence of fluoroquinolone-resistant *Salmonella* (Chitnis et al., 1999; Kapil et al., 1999). CDC estimated a minimum of 100 000 cases of illness due to MDR NTS including approximately 40 deaths annually while 3 800 reported cases of illness caused by MDR *Salmonella* Typhi (CDC, 2013a) in the USA. The trend of increase in the percentage of MDR *Salmonella* isolated in Malaysia is also shown in the National Surveillance of Antibiotic Resistance (NSAR) Report (IMR, 2014).

2.3.2 *Vibrio* spp.

Vibrio spp. is a member of Vibrionaceae. Vibrios are the natural inhabitants of estuarine, marine and brackish water. It blooms when the water is warm (Holmberg, 1988). Not all members of Vibrios are human pathogens, so as foodborne pathogens. *Vibrio cholerae* (*V. cholerae*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *Vibrio vulnificus* (*V. vulnificus*) are the significant public health threats. *V. vulnificus* is able to cause invasive wound infection; *V. cholerae* can cause serious gastroenteritis while *V. parahaemolyticus* can cause both manifestations.

Consumption of raw or undercooked seafood or contaminated water may lead to *Vibrio*-associated-gastroenteritis. Often, the gastroenteritis symptoms are mild but delayed medical treatment could result in the fatal incident (CDC, 2014a). Patients with liver disease, diabetes, and immunocompromised condition are more vulnerable to gastroenteritis. Even though it is invasive, but it is preventable by improving sanitation, especially proper hand washing (Curtis & Cairncross, 2003).

Cholera is the second leading communicable disease in Malaysia (MOH, 2007). The incidence of cholera has been steadily decreasing since the year 2013 (MOH, 2013, 2014a, 2015).

Most of the cholera outbreaks are caused by *V. cholera* O1 El Tor. *V. cholerae* secretes cholera enterotoxin (CT) to allow the adherence to intestinal epithelial cells and increase the production of cyclic adenosine 5-monophosphate (cAMP), eventually trigger the massive secretion of water and electrolytes from the host (Finkelstein, 1996).

Tetracycline has been the first-line drug against cholera. Alternatively, ciprofloxacin, doxycycline, and co-trimoxazole can be used to treat cholera due to the emergence of tetracycline-resistant *V. cholerae* (Krauss et al., 2003).

2.3.3 *Escherichia coli*

Most of the coliforms are harmless to human. The presence of coliforms indicates the faecal contamination and soil or organic matter contamination. *Escherichia coli (E. coli)* is the most common faecal coliform and the most studied member of Enterobacteriaceae. It is present in human gut as part of the microbiota. The presence of *E. coli* in foods and beverages may increase the risk of causing diseases. Thus, *E. coli* is the best indicator for sanitary quality of food and drinks (Edberg et al., 2000; Odonkor & Ampofo, 2013).

Pathogenic *E. coli* can be divided into several categories: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), shigatoxin-producing *E. coli* (STEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and uropathogenic *E. coli* (UPEC). All pathogenic *E. coli* except UPEC are considered as diarrhoeagenic *E. coli*. They could be transmitted by faecal-oral route.

Enterohaemorrhagic *E. coli and* verocytotoxic *E. coli* are categorised under STEC. *E. coli* O157:H7 is a notorious EHEC worldwide as it causes most of the *E. coli* outbreaks. Many outbreaks of *E. coli* O157:H7 are associated with raw beef and RTE salads (CDC, 2013b). Infection of *E. coli* O157:H7 could develop life-threatening haemolytic uremic syndrome (HUS), eventually cause renal failure. Approximately 52% of the transmission of *E. coli* O157 was foodborne, and 14% was due to person-to-person transmission (Rangel et al., 2005). The outbreak cases in Malaysia is not known as it is not documented officially by MOH.

Multidrug-resistant (MDR) *E. coli* strains are not rare. It has consistently been spread in the community (Collignon, 2009). The widespread of antibiotic usage in poultry farming is likely the major contributing factor of MDR *E. coli* in the poultry as well as in humans (Collignon, 2009; Johnson et al., 2007). Spreading of MDR *E. coli* is more severe in the developing countries due to lack of monitoring, resources and control (Kennedy et

al., 2008; Laupland et al., 2008). The import and export of food products also fasten up the spreading of MDR strains (Warren et al., 2008).

The antibiotic resistance genes are usually carried by the mobile genetic elements: transposons, plasmids, and integrons. Horizontal gene transfer (HGT) jeopardises the incidence of antimicrobial resistance infection (Woodford et al., 2011). Moreover, HGT of antibiotic resistance is possible on the abiotic surfaces (Warnes et al., 2012).

2.3.4 *Staphylococcus aureus*

Staphylococcus aureus (*Staph. aureus*) is a natural microbiota found on human skin and nostrils. Usually, it causes no harm to host unless it is present in food up to a certain number of cells (Schelin et al., 2011). It can persist in extremely harsh conditions (Adams & Moss, 2008). Therefore, they can grow in wide range of foods.

Staphylococcal food poisoning (SFP) occurs due to the intoxication of staphylococcal enterotoxins (SEs). SEs are not produced when the bacteria are ingested (Schelin et al., 2011). Although the SE productions are not always associated with *Staph. aureus* growth, but *Staph. aureus* able to produce SEs when its cell density reaches 5-8 Log CFU of bacteria (Lindqvist et al., 2002). The threshold of enterotoxins concentration to trigger SFP is 20-100 ng (Asao et al., 2003). There are 21 SEs identified (Table 2.1). Unlike other bacteria, SEs are relatively heat stable and resist to enzyme degradation. Therefore, *Staph*. *aureus* remains infectious in the human digestive tract even though it is in high acidic condition. (Le Loir et al., 2003).

Table 2.1: Staphylococcal enterotoxins and its general properties

Enterotoxin	Molecular weight (kDa)	Emetic activity	Genetic backbone
SEA	27.1	yes	Prophage
SEB	28.3	yes	SaPI
SEC	27.5- 27.6	yes	SaPI
SED	26.4	yes	Plasmid
SEE	26.4	yes	*egc*
SEG	27.0	yes	Prophage, *egc*
SEH	25.2	yes	scc
SEI	24.3	weak	*egc*
SEIJ	28.6	nk	Plasmid
SEIK	25.5	nk	Prophage, SaPI
SEIL	24.6	no[a]	SaPI
SEIM	24.8	nk	*egc*
SEIN	26.1	nk	*egc*
SEIO	26.7	nk	*egc*
SEIP	27.0	no[a]	Prophage
SEIQ	25.2	nk	SaPI
SER	27.0	yes	Plasmid
SES	26.2	yes	Plasmid
SET	22.6	weak	Plasmid
SEIU	27.1	nk	*egc*
SEIV	nk	nk	*egc*
TSST	15.2	nk	SaPI

nk, not known; SaPI, *Staph. aureus* pathogenicity island; *egc*, enterotoxin gene cluster; no[a], emetic activity were not done in a primate model. (Adapted from Argudin et al, 2008 and Schelin et al., 2011)

The classical enterotoxins are SEA-SEE, also known as prophage-encoded enterotoxins (Schelin et al., 2011). Under a stress condition, the prophage will be induced to replicate and release new bacteriophages (Wallin-Carlquist et al., 2010). SEA and SEE have 90% amino acids similarity (Fraser & Proft, 2008). On the other hand, the SEB, SEC, and SED are known as agr-regulated enterotoxins (Schelin et al., 2011). The production of SEB-SED is a quorum sensing system which enables *Staph. aureus* to respond to its cell density (Thoendel et al., 2011). While SEG-SEIV are non-classical enterotoxins (Schelin et al., 2011). The role of these enterotoxins still remain unclear, but only SEH has been reported to cause SFP (Ikeda et al., 2005).

Penicillin-resistant *Staph. aureus* was first discovered in the 1940s (Rammelkamp & Maxon, 1942), followed by methicillin resistance in 1960s (Jevons, 1961), fluoroquinolone resistance in 1980s (Hooper, 2002), and vancomycin resistance in earlier 2000s (CDC, 2002). The emergence of methicillin resistant *Staph. aureus* (MRSA) was worst case scenario as the coexistence of multi drugs resistance had jeopardised effect of treating the MRSA infections. Moreover, the coexistence of *pvl* genes had increased the virulence of the *Staph*. *aureus* (Appelbaum, 2007).

2.4 Dissemination routes of foodborne pathogens

2.4.1 Ready-to-eat foods

RTE food is defined as food that is ready for consumption at the point of sale; it could be cooked or uncooked (FDA, 2009). Nowadays, more people are spending less time to prepare meals at home because of their inflexibility of working and schooling hour. Therefore, RTE foods have been gaining public favour recently due to its convenience. Street vendors and cafeterias are among the most common sources of RTE foods. It is not only a convenient source of foods, but it also plays a vital role in developing the economy. Street foods and cafeteria foods are considered as low-cost meals. In Malaysia, the annual sales amount of the street foods was estimated at 2.2 billion USD (Winarno & Allain, 1990). However, the food safety and hygiene of the RTE foods are always questionable.

Several pathogens have frequently been reported causing illnesses in particular types of RTE foods. Table 2.2 shows the association of foodborne pathogens in specific food types.

Table 2.2: Foodborne pathogens and the associated foods.

Foodborne pathogens	Associated foods
Salmonella spp.	Egg products, poultry, undercooked or raw meat, unpasteurized milk, raw vegetables, and fruit juices
V. cholerae	Contaminated drinking water and raw shellfish
V. parahaemolyticus	Raw or undercooked seafood
E. coli	Raw vegetables, undercooked beef, juices, and unpasteurised milk
Staph. aureus	Dairy products and salads.
Listeria monocytogenes	RTE smoked seafood, ham, cheese, salads, ice-cream, sausages and unpasteurised milk.
Shigella spp.	Contaminated water and salad.
Campylobacter jejuni	Undercooked poultry, contaminated water, and unpasteurised milk
Yersinia enterocolitica	Undercooked meats and raw milk.

The information above were obtained from Food Safety Inspection and Service (2011) and FDA (2009).

2.4.2 Food handlers

Food handler plays a very important role in food safety. They are involved in almost if not all stages of food preparation from food purchasing. In Malaysia, many foreign food workers are employed to work on the contract basis in the food service industry. Although the government has enforced typhoid vaccination and safe food handling course for all workers involved in food servicing, there are some who do not comply this ruling. Moreover, the medical screening is not mandatory for all food workers. Thus, the background or medical history of these food handlers is unknown. They could be the asymptomatic carriers for foodborne pathogens. A study carried out by Gunn *et al.* (2014) shows the evidence of *Salmonella* being transmitted in an asymptomatic food handler. This situation makes the foodborne illnesses surveillance even more difficult.

Improper hand sanitation by the asymptomatic food handlers could further imperil the consumers as the risk of contamination is even higher. Todd and co-workers (2007) suggested that food handlers could remain as a carrier for 300 days after an episode of infection as they can continuously shed the pathogens of concern. In fact, the improper

food handling by the asymptomatic food handlers had resulted in a food poisoning outbreak in Barcelona, Spain (Barrabeig et al., 2010). Therefore, the attitude and the food handling practices of the food handlers need to be closely monitored.

2.4.3 Food contact surfaces

Food contact surfaces (FCS) like cutting boards, kitchen countertops, cutleries, conveyer belts and packaging surfaces are where the events of bacterial transfer take place. Besides cross contamination, recontamination could occur on the FCS when safe food handling is not practised. Perez-Rodriguez *et al.* (2008) defined recontamination as the event of contamination in food after the inactivation process (such as cooking, dehydration, pasteurisation, and so on).

Salmonella spp., *E. coli*, *Staph. aureus*, *Campylobacter* spp. and *Listeria monocytogenes* are amongst the foodborne pathogens that are always associated with cross contamination of FCS. The bacterial transfer ability is bacteria species-dependent (Knobben et al., 2007; Midelet et al., 2006) which could be due to the difference in adherence characteristics (Perez-Rodriguez et al., 2008). Joseph *et al.* (2001) and Stepanovic *et al.* (2004) reported that *Salmonella* could attach to inert surfaces and form biofilms. On the other hand, *Staph. aureus* can survive longer (up 96 hours) than *S.* Enteritidis and *C. jejuni* on dry surfaces which probably due to its aggregation structure (Kusumaningrum et al., 2003). The foodborne pathogens can remain viable on dry FCS and eventually increase the risk of recontamination in foods.

Cutting board is always perceived as a fomite for foodborne disease transmission, regardless of the materials of the cutting boards. The knife-scarred cutting boards are tough to be disinfected completely. Moreover, the food juices and bacteria could be drawn into the scarred/pores of the cutting board by capillary action (Cliver, 2006). It could be

a perfect habitat for bacteria. However, proper sanitation of the cutting board was proved to decrease the microbial loads effectively (Cliver, 2006).

Besides that, FCS could be an intermediate substance for the bacterial transfer. Several simulation studies carried out by other researchers have shown that bacteria were disseminated from the foods to another food via the FCS, especially the cutting board and knife (Jeyaletchumi et al., 2012; Kusumaningrum et al., 2003). Hence, the retention of foodborne pathogens on FCS has a great impact on foodborne disease transmission.

2.4.4 Table cleaning cloths

Other than FCS, the microbial contamination in table cleaning cloths (TCC) is as important. Inappropriate sanitation of the TCC may lead to cross contamination of FCS and food. *E. coli* O157:H7, *Salmonella* spp. and *Campylobacter* spp. are able to survive during domestic washing-up (Mattick et al., 2003). Moreover, the residual water trapped in the cloths allows the bacteria to survive longer periods and hence, increase the cross contamination events (Bloomfield, 2003). Hence, TCC could be a possible transmission vehicle.

2.5 Characterisation of potential pathogens

2.5.1 Antibiotic resistance profiling

The antibiotic resistance profile varies enormously among the same bacterial species. The antibiotic resistance property of a bacterial strain can be intrinsic or acquired. Broth dilution has always been the gold standard to determine the susceptibility of a bacterial strain towards an antibiotic. But the time-consuming procedure in broth dilution testing leads to the development of disk diffusion method (Hudzicki, 2009). Kirby-Bauer disk diffusion method has been widely adopted in microbiology laboratory (Reller et al., 2009). In additional, Clinical Laboratory Standards Institute (CLSI) is constantly

updating the standard and requirements for this method. Thus, this technique is validated from time-to-time for its relevance.

2.5.2 Virulotyping

Virulotyping in foodborne pathogens is essential to differentiate the virulent and avirulent strains. The conventional virulotyping method involved identification, biochemical, enzymatic and serology tests. However, the conventional virulotyping method is not able to address the unique virulence genes (Gutler et al., 2017). Currently, PCR and microarrays are mostly deployed for virulotyping in food safety testing (Wassenaar, 2011). However, the genome-sequencing is more robust method compared to PCR and microarrays but not as practicable in food safety testing (Gutler et al., 2017). Thus, PCR-based virulotyping is still favoured.

2.5.3 DNA fingerprinting

Pulsed-field gel electrophoresis (PFGE) has been the "gold standard" method for pathogen subtyping since the establishment of PulseNet back in 20 years ago. PulseNet uses PFGE as a source tracking method to investigate the bacteria isolated from outbreaks or even sporadic cases (Chen et al., 2004), foods and environmental samples. PFGE has high discriminative power and high reproducibility (CDC, 2016). The same protocol can be applied to subtype the same bacterial species except that the choice of restriction enzymes is needed to be optimised. On the other hand, this method is time-consuming and requires a skillful handling and strict adherence to standard protocols to obtain reproducible bands. Therefore, PulseNet has been shifting its preference in PFGE to sequence-based typing like Multilocus Sequencing Typing (MLST) and Whole Genome Sequencing (WGS). However, these methods are relatively more expensive than the PCR-based DNA fingerprinting.

PCR-based DNA fingerprinting methods such as Repetitive Extragenic Palindromic (REP)-PCR, Random Amplified Polymorphic DNA (RAPD)-PCR, Enterobacterial Repetitive Intergenic Consensus Sequence (ERIC)-PCR have been widely used to genetically characterised the foodborne pathogens (Jarraud et al., 2002; Navia et al., 1999; Rivera et al., 2001; Singh et al., 2011; Teh et al., 2011). Despite the fact that PCR-based DNA fingerprinting method has always been associated with low discriminatory power and poor reproducibility, the cost of analysis is relatively low, and the protocol is simple. Among the PCR-based fingerprinting methods, REP-PCR is known to be the most discriminative and most reproducible method (Bou et al., 2000; Ishii & Sadowsky, 2009; Lim et al., 2009).

2.6 Hazard identification and hazard characterisation of microbial food safety

The objective of hazard identification is to identify the microbiological hazards (e.g., pathogens or microbial toxins) present in foods. Clinical, prevalence, epidemiological and surveillance studies were the examples to identify hazards.

Hazard characterisation is the qualitative or quantitative, or both description of the severity and duration of the adverse effects to human after ingestion of the pathogen or microbial toxin in food. It could be studied via a dose response assessment. There are several important factors need to be considered for hazard characterisation: virulence factors, genetic materials for horizontal gene transfer, antibiotic resistance, microbial dissemination, microbial persistence after infection, the threshold for infection, and the attributes of food contents (Codex Alimentarius Commission, 2001a).

2.7 Knowledge, attitude and practices of the food handlers with regards to food safety

Knowledge, attitude and practices (KAP) of the food handlers are the three key indicators that may contribute to food safety. The definitions of each key indicator are shown in Table 2.3. Assessing the food safety knowledge, attitude and practices allow us to better understand the food handling situation so as to provide us with an insight of the social, psychological and behavioural factors (Macías & Glasauer, 2014). It helps to identify the misconceptions and misunderstandings that may be the barrier to implementing a change (Monde, 2011). Hence, the authority can focus on the weaknesses or the barriers identified from KAP study to strengthen the food safety measures.

Table 2.3 Definitions of each key indicator.

Knowledge	The understanding of a given subject. (Kaliyaperumal, 2004)
Attitudes	Emotional, motivational, perceptive and cognitive beliefs that may positively or negatively affect a personnel's practices and behaviour. (Andrien, 1994; De Landsheere, 1982)
Practices	The observable actions of a personnel.

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

3.1.1 Media preparation

Two types of pre-enrichment media (buffered peptone water and alkaline peptone water), selective media. All media preparation steps are listed in Appendix A.

3.1.2 Chemical preparation

There were several buffers used in this study. All chemical solutions preparation methods are listed in Appendix B.

3.2 Research framework

This study comprised of 3 different aims: i) microbiological quality assessment, ii) food safety knowledge, attitudes and practices (KAP) assessment, and iii) characterisation of the isolated bacteria. The enumeration, isolation and identification of hygiene indicators, potential pathogens are to assess the microbiological quality of the factors associated with ready-to-eat foods. At the same time, the knowledge, attitudes, self-report practices in regards to food safety and microbiological quality of the food handlers' hands are assessed. The strains obtained from these 2 sections were then characterised according to its antibiotic resistance profile, virulence profile, and genetic diversity by using antimicrobial susceptibility testing (AST), polymerase chain reaction (PCR), REP-PCR and pulsed-field gel electrophoresis (PFGE), respectively.

3.2 Research Framework

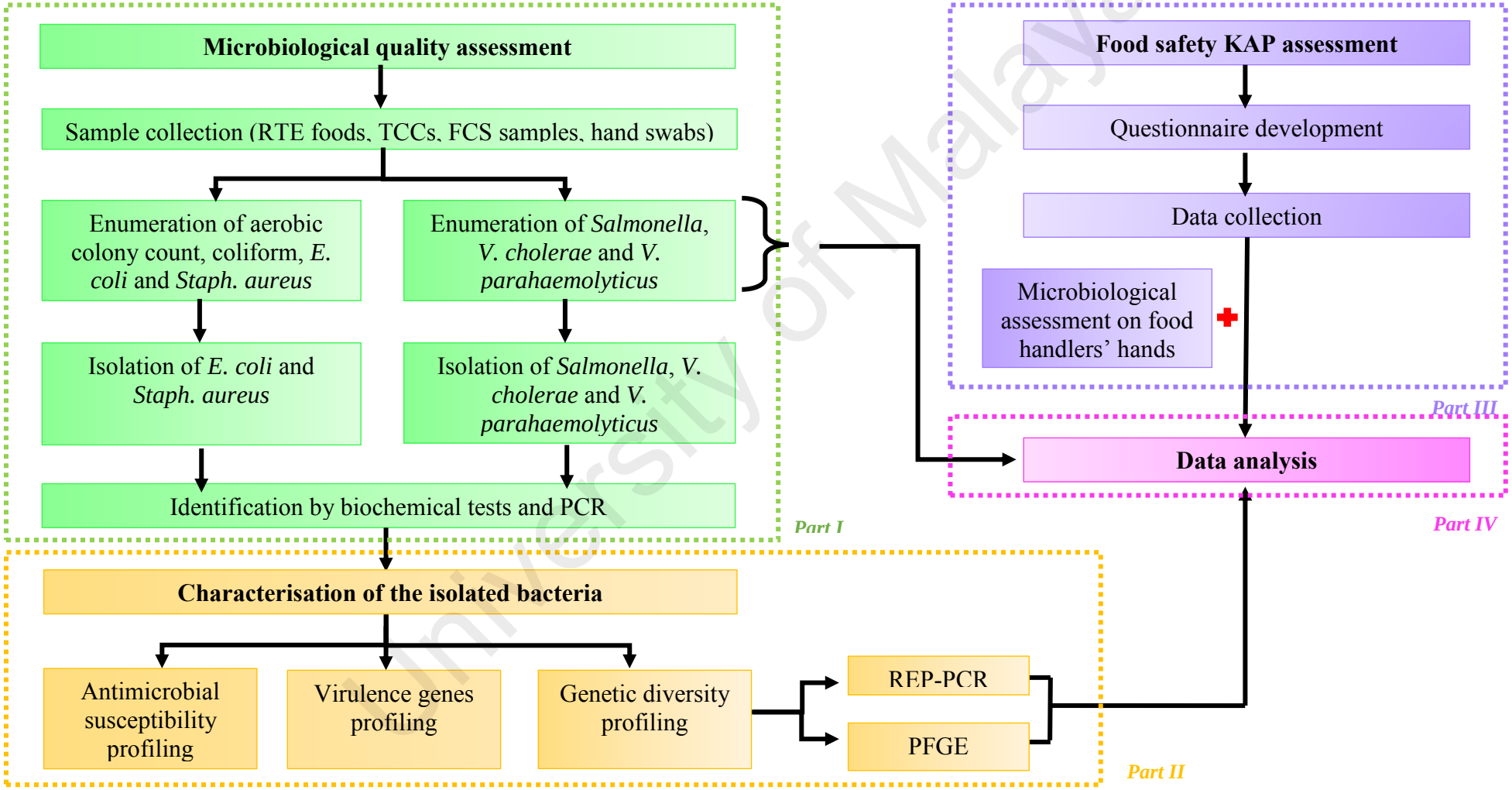


Figure 3.1: Research framework of the study of microbial risk associated with ready-to-eat foods.

3.3 Research method- Microbiological quality assessment (*Part I*)

3.3.1 Sample collection

Sample collection was conducted in a public university located in Kuala Lumpur, and its vicinity. The University accommodates 24000 students and staff according to the 2014 record. It has 25 food premises in the campus, but only 18 food premises serve lunch. Apart from that, students and staff also patronise the restaurants and stalls in the vicinity of the campus.

A total of 150 RTE food samples, 34 table cleaning cloth (TCC) samples, 59 food contact surfaces (FCS) swabs and 85 hand swabs were collected from the food premises in the campus from November 2013 to August 2014, some RTE food samples were also collected from the food premises located in the vicinity of campus. Random sampling was carried out in each food premises.

The 150 RTE foods were categorised into eight different groups as listed in Table 3.1. The food samples were packed in the clean plastic bags or Styrofoam boxes by the food handlers and transferred to the laboratory for immediate analysis.

TCC samples were soaked and pressed firmly in 200 mL maximum recovery diluent (MRD; Merck, Germany) to extract the microbial contaminants on the TCC. On the other hand, FCS swab samples include cutting boards, cutleries, and counter tops. A sterile swab moistens with MRD was used to swab over an area of 100 cm^2 of the FCS samples or the hands of food handlers' hands and dipped into the tube containing 10 mL MRD. All these samples were transported to the laboratory within one hour for immediate microbiological analysis.

Table 3.1: The description of RTE foods according to respective food groups.

Food groups	Description	Number of samples
A	Cooked food for immediate consumption (e.g., Noodle soup, fried rice, *roti canai*)	14
B	Fully cooked food kept warm on a heater or display (e.g., buffet-style dishes, *rendang ayam*)	39
C	Combination of cooked and uncooked RTE foods (e.g., chicken rice, *nasi lemak*, *rojak*, fried rice with raw vegetables as topping)	16
D	Uncooked food that ready for consumption (e.g., sliced fruits, 'ulam')	13
E	Hot drinks with milk (e.g., Hot chocolate drinks, hot coffee (Latte))	17
F	Hot drinks without milk (e.g., Hot coffee (Espresso))	12
G	Cold drinks with milk (e.g., rose syrup with milk, cold chocolate drinks)	23
H	Cold drinks without milk (e.g., cold rose syrup)	16

3.3.2 Enumeration of aerobic colony count, coliforms, *E. coli* and *Staph. aureus*

In this study, Petrifilm™ (3M™, USA) Aerobic Count Plate, Petrifilm™ *E. coli*/coliform and Petrifilm™ Staph Express Count Plate were chosen to determine the aerobic colony count, coliforms, *E. coli,* and *Staph. aureus* count, respectively. The RTE food samples (10 g) were homogenised with 90 mL of MRD using a stomacher (Stomacher Lab, UK) for 2 min. Then, the suspensions of the homogenate, TCC extracts, liquid from FCS swabs and hand swabs were used for dilutions up to 10^{-3}. Depending on the microbiological criteria as listed by Gilbert *et al.*, (2010), Sneed *et al*., (2004) and Willis *et al*., (2013), different dilutions was inoculated onto the Petrifilm™ to determine the satisfactory level of the bacterial counts. The inoculated Petrifilms™ were then incubated and interpreted as according to the interpretation guides.

3.2.3 Isolation of *E. coli* and *Staph. aureus*

E. coli appeared as blue colony surrounded by air bubble on the Petrifilm™ *E. coli*/coliform Count Plate. These colonies were picked and streaked on CHROMagar Orientation (CHROMagar, France) for purification purpose if needed. Purple colonies on the CHROMagar are the presumptive *E. coli*. The isolates were transferred to the Luria-Bertani Agar (LBA; BD, USA) for preservation and further identification.

The red-violet isolates on the Petrifilm™ Staph Express Count Plate were identified as the presumptive *Staph aureus*. The colony was streaked on a mannitol salt agar (MSA; Oxoid) when purification was needed. The presumptive *Staph. aureus* was preserved in tryptic soy agar (TSA; Oxoid).

3.3.4 Enumeration *Salmonella* spp., *V. cholerae* and *V. parahaemolyticus*

The modified protocols from Bacteriological Analytical Manual (Andrews & Hammack, 2003) were referred. To enumerate *Salmonella*, *V. cholerae*, and *V. parahaemolyticus*, 3-tube MPN-PCR was applied. Ten grammes of RTE food samples were homogenised with 90 mL of buffered peptone water (Merck) for 1 min to enumerate and isolate *Salmonella*. Then, the homogenate and the transport medium for TCC, FCS and hand swab samples were diluted up to 10^{-3}. Diluted samples were incubated at 35±2°C for 18- 24 hours. After incubation, 0.1 mL of the overnight culture was transferred to a new microcentrifuge tube containing 0.9 mL selenite cysteine broth (Oxoid, UK) and incubated at the same condition.

To enumerate *V. cholerae* and *V. parahaemolyticus*, 10 g of food were weighed and stomached with 90 mL of alkaline peptone water (Merck). The food homogenate and the transport medium of other samples were diluted to 10^{-3} and incubated at 35 ± 2°C for overnight.

The overnight culture was then spun down and washed three times with 1 × TE buffer. A hundred microliter of sterile ddH_2O was added to re-suspend the pellet. Then, it was boiled at 99°C for 10 min and instantly chilled on ice. The lysate was then centrifuged at 13400 rpm. The supernatant was used as PCR template. The PCR conditions and master mixes to enumerate *Salmonella*, *V. cholerae,* and *V. parahaemolyticus* are summarised in Table 3.3.

After PCR, the PCR products were electrophoresed in 1.5% analytical grade agarose (Promega, USA) for 30 min under 100V. Then the gel was stained with Gel Red (Biotium, USA) for 20 min and visualised under UV ray equipped with a Gel documentation (Bio-Rad, USA).

Based on the presence of targeted DNA on the gel, the number of positive tubes according to the dilution factor was recorded. Then, the MPN value was obtained by inserting the results into MPN calculator (Curiale, 2014).

Table 3.2: Microbial assessment criteria used to interpret the results.

	Thresholds for the specific sample type			
	RTE foods[c]	**FCS[d]**	**TCC[e]**	**Food handlers[f]**
Hygiene indicators[a]				
Aerobic colony count	4.0 log	1.3 log	4.0 log	1.3 log
Total coliforms	4.0 log	1.3 log	4.0 log	1.3 log
E. coli	2.0 log	1.0 log	2.7 log	1.0 log
Staph. aureus	2.0 log	1.0 log	2.7 log	1.0 log
Foodborne pathogens[b]				
Salmonella spp.	N. D.	N/A	N/A	N/A
V. cholerae	N. D.	N/A	N/A	N/A
V. parahaemolyticus	3.0 log	N/A	N/A	N/A

N.D., not detected; N/A, not available.
[a] The hygiene indicators were recorded for percentage of satisfactory and acceptable samples based on guidelines referred.
[b] The number of samples detected positive for the foodborne pathogens based on MPN-PCR method.
[c] The threshold is in a unit of CFU/g or MPN/g , referred from Gilbert *et al.,* 2010.
[d] The threshold is in a unit of CFU/cm^2 or MPN/ 100 cm^2, referred from Sneed *et al*., 2004
[e] The threshold is in a unit of CFU/cloth or MPN/cloth referred from Willis *et al*., 2013.
[f] The threshold is in a unit of CFU/cm^2 or MPN/hand referred from Sneed *et al.,* 2004 & Tan *et al.,* 2013.

Table 3.3: PCR conditions and primers used for MPN-PCR.

Target bacteria	Primer name	Primer sequence 5'-->3	Target gene	Amplicon size (bp)	PCR condition	Reference
***Salmonella* spp.**	OMPC F[1]	ATCGCTGACTTATGCAATCG	*ompC*	204	Initial denaturation: 95°C 2 min Denaturation: 95°C 1 min Annealing: 55 °C 1 min Extension: 72°C 2 min, 35 cycles, Final extension: 72°C 5 min.	(Alvarez et al., 2004)
	OMPC R[1]	CGGGTTGCGTTATAGGTCTG				
V. cholerae	pntA 1C[2]	CAGTAAAGAAACGACCAAA CTC	*pntA*	338	Initial denaturation: 95°C 3 min Denaturation: 94°C 30 sec Annealing: 59 °C 30 sec Extension: 72°C 1 min, 35 cycles, Final extension: 72°C 5 min.	(Teh et al., 2010)
	pntA 2C[2]	TGCCAGTTTCGATGATGCCG				
V. parahaemolyticus	pntA 1P[2]	AGCAAGTTTCGATGATGCTG	*pntA*	409		
	pntA 2P[2]	ACCAGCAACCAAAACTTTCG CT				

[1]The master mix (20µL per reaction) consisted of 1X Buffer,1.5 mM, $MgCl_2$, 0.28 µM dNTPs, 0.4 µM primers and 1U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template.
[2]The master mix (20µL per reaction) consisted of 1X Buffer, 2mM $MgCl_2$, 0.2mM dNTPs, 0.5µM primers and 1U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template.

3.3.5 Validation of MPN-PCR results

The representative remaining PCR products after gel electrophoresis were sent for sequencing at the commercial laboratory (First BASE Laboratory Sdn. Bhd.). After sequenced, the nucleotides sequence were blast against National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

3.3.6 Isolation of *Salmonella* spp., *V. cholerae* and *V. parahaemolyticus*

From the MPN tubes that yielded positive results in the PCR detection, a loopful of the selectively enriched culture was streaked on CHROMagar *Salmonella* & XLD, CHROMagar *Vibrio* & TCBS for the isolation of *Salmonella* and Vibrios, respectively. Then, the presumptive isolates were transferred onto LBA for preservation, further identification and characterisation.

3.3.7 Identification of bacterial isolates

The presumptive isolates were tested for oxidase test by smearing the cells on the OxiStrips (Hardy Diagnostics, USA) and 3% KOH string test to eliminate the false positive identification. *E. coli* ATCC® 25922™ was used as the negative control while *V. cholerae* O1 N16961 was used as the positive control in oxidase test. In the string test, *Staph. aureus* ATCC® 25923™ was chosen as the negative control while *E. coli* ATCC® 25922™ was the positive control. The presumptive *V. cholerae* isolates were biotyped by *V. cholerae* Antisera (Denka Seiken, Japan). After primary identification, the isolates were further confirmed by either species-specific PCR. The PCR conditions and the master mixes are listed in Table 3.4 while the PCR identification conditions and primers for *Salmonella*, *V. cholerae* and *V. parahaemolyticus* were tabulated in Table 3.3.

Table 3.4: Primers used for identification of specific bacteria and its condition.

Target bacteria	Primer name	Primer sequence 5'-->3	Target gene	Amplicon (bp)	PCR condition	Reference
E. coli	Pho-F	GTGACAAAAGCCCGGACACCATAAATGCCT	*pho*A[1]	903	Initial denaturation: 95°C, 5 min Denaturation: 96°C, 1 min Annealing: 52 °C, 30 sec Extension: 72°C, 1 min, 30 cycles Final extension: 72°C, 10 min	(Kong et al., 1999)
	Pho-R	TACACTGTCATTACGTTGCGGATTTGGCGT				
Staph. aureus	Nuc F	GCGATTGATGGTGATACGGT	*nuc*A[2]	267	Initial denaturation: 94°C, 2 min Denaturation: 94°C, 1 min Annealing: 55 °C, 30 sec Extension: 72°C, 90 sec, 35 cycles Final extension: 72°C, 4 min.	(Brakstad et al., 1992)
	Nuc R	AGCCAAGCCTTGACGAACTAAAGC				
	MecA F	GTAGAAATGACTGAACGTCCGATAA	*mec*A[1]	310	Initial denaturation: 95°C, 5 min Denaturation: 96°C, 1 min Annealing: 52 °C, 30 sec Extension: 72°C, 1 min, 30 cycles Final extension: 72°C, 10 min	(Geha et al., 1994)
	MecA R	CCAATTCCACATTGTTTCGGTCTAA				

[1] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5µM primers and 1U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template.
[2] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4µM primers and 1U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template.

3.4 Research method- Bacterial characterisation (*Part II*)

3.4.1 Antimicrobial susceptibility profiling

The antimicrobial profile of the isolated bacteria was determined by using Kirby-Bauer disc diffusion technique based on the standard set by Clinical Laboratory Standards Institute (2015). The isolates were freshly cultured on the non-selective agar before the experiment. By using Dade Microscan turbidity meter (Baxter Diagnostics, Inc., McGraw Park, III), the cell density of 0.06 (shown in the turbidity meter) was obtained and swabbed evenly on a Muller-Hinton II agar (BD). The inoculated plates were then incubated according to the standard. One or two relevant antibiotics were chosen from each drug group stated in CLSI. Based on literature review, these antibiotics are repeatedly associated with resistance. All antibiotics tested on the respective bacteria are tabulated in Table 3.5.

3.4.2 Virulence gene profiling

DNA was isolated freshly from the bacterial colonies by using the boiling method and kept at -20°C. Different virulence genes were tested on each bacterial species. The virulence genes chosen in this study were highly associated with the foodborne pathogens studied based on literature review. Table 3.6 shows the virulence gene tested and its PCR condition. The virulence genes detection for *Staph. aureus* isolates were carried out in 7 multiplex PCR which has been denoted in Table 3.6. The presence of respective virulence genes was determined by the presence of DNA band on the 2% LE agarose (Promega) after electrophoresis the PCR products.

Table 3.5: The antibiotics that tested on the specific bacteria.

Bacteria	Drug class	Antibiotic tested (concentration, µg)	Reference
E. coli	Aminoglycosides	Gentamycin (10), Kanamycin (30), Streptomycin (15)	(Collignon et al., 2009; Johnson et al., 2007; Lim et al., 2009)
	Penicillin	Ampicillin (10)	
	Cephems	Ceftriaxone (30), Cefuroxime (30)	
	Carbapenems	Imipenem (10), Meropenem (10)	
	Quinolones	Nalidixic acid (30), Ciprofloxacin (5)	
	Tetracyclines	Tetracycline (30)	
	β-lactam	Amoxicillin-Clavulanic acid (25)	
	Folate pathway inhibitor	Trimethoprim-sulfamethoxazole (25)	
	Phenicols	Chloramphenicol (30)	
	Macrolides	Erythromycin (15)	
Staph. aureus	Penicillinase-labile Penicillins	Penicillin (10U)	(IMR, 2014; Lim et al., 2012)
	Glycopeptides	Teicoplanin (30)	
	Aminoglycosides	Gentamycin (10), Kanamycin (30)	
	Macrolides	Erythromycin (15)	
	Quinolones	Ciprofloxacin (5)	
	Tetracycline	Tetracycline (30)	
	Lincosamides	Clindamycin (2)	
	Folate pathway inhibitor	Trimethoprim-sulfamethoxazole (25)	
	Phenicols	Chloramphenicol (30)	
	Ansamycin	Rifampicin (5)	
	Oxazolidone	Linezolid (30)	
V. cholerae & V. parahaemolyticus	Tetracycline	Tetracycline (10)	(Paydar, et al., 2013; Teh et al., 2012)
	Carbapenems	Ertapenem (10), Imipenem (10)	
	β-lactam	Amoxicillin-Clavulanic Acid (25)	
	Folate pathway inhibitor	Trimethoprim-sulfamethoxazole (25)	
	Quinolones	Nalidixic acid (30), Ciprofloxacin (5)	
	Phenicols	Chloramphenicol (30)	
	Macrolides	Erythromycin (15), Azithromycin (15)	
	Nitrofurans	Furazolidone (100mg)	
	Cephems	Ceftriaxone (30), Cefazolin (30)	
	Monobactam	Aztreonam (30)	

3.4.3 Genetic diversity profiling (Repetitive extragenic palindromic - polymerase chain reaction, REP-PCR)

The bacterial culture was freshly cultured in the tryptic soy broth (TSB; Oxoid) overnight at 35 ± 2°C. On the next day, DNA was isolated using the method mentioned in Section 3.1.2. There were two different primers used to study the genetic diversity of *E. coli*, *Staph. aureus, V. cholerae* and *V. parahaemolyticus*. The primers used and the condition of PCR are summarised in Table 3.7.

PCR products were then loaded into a solidified 120 mL 1.5% LE agarose (Promega) for electrophoresis (100V, 5 ½ h). After electrophoresis, the gel was stained with Gel Red (Biotium) for 30 min and then view under UV ray equipped with a Gel documentation system (Bio-Rad).

Table 3.6: Primers used for virulotyping for each specific bacterium.

Primer	Primer sequence (5'→ 3')	Virulence gene	Amplicon size (bp)	Reference
E. coli				
ST1-F	CTTTCCCCTCTTTTAGTCAG	ST1	175[α]	(Kong et al., 1999)
ST1-R	TAACATGGAGCACAGGCAGG			
LT1-F	TTACGGCGTTACTATCCTCTCTA	LT1	275[α]	
LT1-R	GGTCTCGGTCAGATATGTGATTC			
LT2-F	ATATCATTTTCTGTTTCAGCAAA	LT2	720[α]	
LT2-R	CAATAAAATCATCTTCGCTCATG			
VT-F	GAACGAAATAATTTATATGTG	VT1 & VT2	523 and 520[α]	
VT-R	CCTGATGATGGCAATTCAGTA			
AE22	ATTACCATCCACACAGACGGT	*eae*A	4397[α]	(Fratamico & Strobaugh, 1998)
AE20-2	ACAGCGTGGTTGGATCAACCT			
Staph. aureus				
cna F	AGTGGTTACTAATACTG	*cna*[C]	744[β]	(Kumar et al., 2009)
cna R	CAGGATAGATTGGTTTA			
icaA F	GATTATGTAATGTGCTTGGA	*ica*A[F]	770[γ]	
icaA R	ACTACTGCTGCGTTAATAAT			
sdrE F	AGTAAAATGTGTCAAAAGA	*sdr*E[G]	767[γ]	
sdrE R	TTGACTACCAGGCTATATC			
hlg F	GCCAATCCGTTATTAGAAAATGC	*hlg*[C]	937[γ]	
hlg R	CCATAGACGTAGCAACGGAT			
SEA-1	GAAAAAAGTCTGAATTGCAGGGAACA	*sea*[A]	560[β]	(Jarraud et al., 2002)
SEA-2	CAAATAAATCGTAATTAACCGAAGGTTC			
SEB-1	ATTCTATTAAGGACACTAAGTTAGGGA	*seb*[A]	404[β]	

Table 3.6, continued.

SEB-2	ATCCCGTTTCATAAGGCGAGT			
mpSEC-1	GTAAAGTTACAGGTGGCAAAACTTG	*sec*[A]	297[β]	
mpSEC-2	CATATCATACCAAAAAGTATTGCCGT			
SED-1	GAATTAAGTAGTACCGCGCTAAATAATATG	*sed*[A]	492[β]	
SED-2	GCTGTATTTTTCCTCCGAGAGT			
SEE-1	CAAAGAAATGCTTTAAGCAATCTTAGGC	*see*[E]	482[β]	
SEE-2	CACCTTACCGCCAAAGCTG			
SEG-1	AATTATGTGAATGCTCAACCCGATC	*seg*[B]	642[β]	
SEG-2	AAACTTATATGGAACAAAAGGTACTAGTTC			
SEH-1	CAATCACATCATATGCGAAAGCAG	*seh*[B]	376[β]	
SEH-2	CATCTACCCAAACATTAGCACC			
SEI-1	CTCAAGGTGATATTGGTGTAGG	*sei*[B]	576[β]	
SEI-2	AAAAAACTTACAGGCAGTCCATCTC			
mpSEJ-1	TAACCTCAGACATATATACTTCTTTAACG	*sej*[B]	300[β]	
mpSEJ-2	AGTATCATAAAGTTGATTGTTTTCATGCAG			
TST-1	TTCACTATTTGTAAAAGTGTCAGACCCACT	*tst*[E]	180[β]	
TST-2	TACTAATGAATTTTTTTATCGTAAGCCCTT			
mpETA-1	ACTGTAGGAGCTAGTGCATTTGT	*eta*[D]	190[β]	
mpETA-3	TGGATACTTTTGTCTATCTTTTTCATCAAC			
mpETB-1	CAGATAAAGAGCTTTATACACACATTAC	*etb*[D]	612[β]	
mpETB-2	AGTGAACTTATCTTTCTATTGAAAAACACTC			
ET-14	AACTATCATGTATCAAGG	*etd*[D]	376[β]	
ET-15	CAGAATTTCCCGACTCAG			
efb-1	AAC ATT AGC GGC AAT AGG	*efb*[C]	432[β]	

Table 3.6, continued.

efb-2	ATT CGC TCT TGT AAG ACC			(Colque-Navarro et al., 2000)
fnbA-1	GATACAAACCCAGGTGGTGG	*fnb*A[F]	191[γ]	(Arciola et al., 2005)
fnbA-2	TGTGCTTGACCATGCTCTTC			
fnbB-1	TGTGCTTGACCATGCTCTTC	*fnb*B[G]	201[γ]	(Lina et al., 1999)
fnbB-2	AGTTGATGTCGCGCTGTATG			
luk-PV-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	*pvl*[F]	433[γ]	
luk-PV-2	GCATCAASTGTATTGGATAGCAAAAGC			
V. cholerae				
CTX7	GGTTGCTTCTCATCATCGAACCAC	*ctx*B	461[δ]	(Olsvik et al., 1993)
CTX9B	GATACACATAATAGAAITAAGGATG			
rtxC-F	CGACGAAGATCATTGACGAC	*rtx*C	263[δ]	(Chow et al., 2001)
rtxC-R	CATCGTCGTTATGTGGTTGC			
tcpA-F	CACGATAAGAAAACCGGTCAAGAG	*tcp*A	453 (ET); 620 (Cla)[δ]	(Singh et al., 2002)
tcpA-B/ Class	TTACCAAATGCAACGCCGAATG			
tcpA-B/ El	CGAAAGCACCTTCTTTCACACGTTG			
132F	TAGCCTTAGTTCTCAGCAGGCA	*tcp*I	862[δ]	(Rivera et al., 2001)
951R	GGCAATAGTGTCGAGCTCGTTA			
rstRclaF	TTTGCTACTTCTTCTTGGTT	*rst*R	887 (ET); 732 (Cla)[δ]	(Kimsey et al., 1998)
rstRETF	TGAGCATAAGCTCTTGATTT			
rstAR	CCGTGAAAGTCATCAACG			
hlyA1	GTGCGTATCAGCCTAGATGA	*hly*A	255 (ET); 244 (Cla)[δ]	(Teh et al., 2009)
hlyA2	CCCAAGCTCAAAACCTGAAA			
zotF	TCGCTTAACGATGGCGCGTTTT	*zot*	947[ε]	(Singh et al., 2002)
zotB	AACCCCGTTTCACTTCTACCCA			
101F	CCTTCGATCCCCTAAGCAATAC	*tox*R	779[ε]	(Olsvik et al., 1993)

Table 3.6, continued.

837R	AGGGTTAGCAACGATGCGTAAG			
94F	CGGGCAGATTCTAGACCTCCTG	*ctx*A	564[ε]	(Rivera et al., 2001)
614R	CGATGATCTTGGAGCATTCCCAC			
ompW2	GAACTTATAACCACCCGCG	*omp*W	336[θ]	(Nandi et al., 2000)
ompW3	CCACCTACCTTTATGGTCC			
O1rfbF	GTTTCACTGAACAGATGGG	*rfb*	192[θ]	(Hoshino et al., 1998)
O1rfbR	GGTCATCTGTAAGTACAAC			

[A,B,D,D,E,F & G] The multiplex PCR A, B, C, D, E, F, and G are denoted at the column of 'virulence gene'.

[α.] This amplicon was amplified in a master mix (45 µL per reaction) consisted of 1X buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.7µM STI primers, 0.7µM *eae* primers, 0.7µM LT1 primers, 6.0 µM LT2 primers, 7.0µM VT primers and 1.5U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template; under such condition: Initial denaturation: 94°C 2 min, Denaturation: 94°C 1 min, Annealing: 56 °C 1 min, Extension: 72°C 1 min, 35 cycles, Final extension: 72°C 10 min.

[β] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.8 mM $MgCl_2$, 0.35 mM dNTPs, 0.3µM primers and 1.5U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template; under such condition: Initial denaturation: 95°C 5 min, Denaturation: 95°C 1 min, Annealing: 55 °C 1 min, Extension: 72°C 1 min, 30 cycles, Final extension: 72°C 5 min.

[γ] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.4 mM $MgCl_2$, 0.35 mM dNTPs, 0.3µM primers and 1.5U *Taq*, ≈50 ng of DNA (5µL) was needed as DNA template; under such condition: Initial denaturation: 95°C 5 min, Denaturation: 95°C 1 min, Annealing: 55 °C 1 min, Extension: 72°C 1 min, 30 cycles, Final extension: 72°C 5 min.

[δ] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.0 mM $MgCl_2$, 0.80 mM dNTPs, 0.1µM primers and 1.0U *Taq*, ≈20 ng of DNA (5µL) was needed as DNA template; under such condition: Initial denaturation: 95°C 2 min, Denaturation: 95°C 30 sec, Annealing: 57 °C 30 sec, Extension: 72°C 40 sec, 30 cycles, Final extension: 72°C 5 min.

[ε] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.2 mM $MgCl_2$, 0.30 mM dNTPs, 0.3µM primers and 1.0U *Taq*, ≈20 ng of DNA (5µL) was needed as DNA template; under such condition: Initial denaturation: 95°C 5 min, Denaturation: 95°C 30 sec, Annealing: 59 °C 30 sec, Extension: 72°C 1 min, 30 cycles, Final extension: 72°C 7 min.

[θ] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.2 mM $MgCl_2$, 0.30 mM dNTPs, 0.3µM primers and 1.0U *Taq*, ≈20 ng of DNA (5µL) was needed as DNA template; under such condition: Initial denaturation: 95°C 5 min, Denaturation: 95°C 30 sec, Annealing: 59 °C 30 sec, Extension: 72°C 1 min, 30 cycles, Final extension: 72°C 7 min.

Table 3.7: Primers used in REP-PCR.

Primer	Primer sequence (5'→ 3')	Bacteria	PCR condition	Reference
REP[a]	GCG CCG ICA TGC GGC ATT	*E. coli*	Initial denaturation: 94°C 7 min Denaturation: 94°C 30 sec Annealing: 44 °C 1 min Extension 72°C 8 min, 30 cycles, Final extension: 72°C 16 min	(Navia et al., 1999), (Lim et al., 2009; Teh et al., 2011)
REP[a]	GCG CCG ICA TGC GGC ATT	*V. cholerae*	Initial denaturation: 94°C 4 min Denaturation: 94°C 31 min Annealing: 42 °C 1 min Extension: 68°C 8 min , 35 cycles , Final extension: 72°C 8 min.	
RW3A[b]	TCGCTCAAAACAACGACACC	*Staph. aureus*	Initial denaturation: 95°C 3 min Denaturation: 94°C 1 min Annealing: 54 °C 2 min, Extension: 72°C 2 min, 35 cycles, Final extension: 72°C 5 min.	(Vecchio et al., 1995)

[a] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.6µM primers and 1.0U *Taq*, ≈200 ng of DNA (5µL) was needed as DNA template.
[b] This amplicon was amplified in a master mix (20 µL per reaction) consisted of 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.6µM primers and 1.0 U *Taq*, ≈200 ng of DNA (5µL) was needed as DNA template.

3.4.4 Genetic diversity profiling (Pulsed-field gel electrophoresis, PFGE)

In each PFGE gel, *Salmonella* Braenderup H9812 which is the universal size standard strain was run together with the strains that needed to be characterised. All PFGE gel was electrophoresed using CHEF-Mapper® XA system (Bio-Rad). Before cell suspension preparation, a 20 mL 1% Seakem Gold agarose (Cambrex Bio Science Rockland, USA) was dissolved in 1×TE buffer and kept in water bath at 50°C. A 2 mL cell suspension buffer was prepared in a 5 mL clear falcon to measure the turbidity by Dade Microscan turbidity meter (Baxter Diagnostics, Inc.). The overnight (16-18 hours) culture was swabbed by using a sterile cotton swab that moistens in cell suspension buffer to obtain the specific density of cells.

PFGE for Gram-negative bacteria (*E. coli V. cholerae* and *Salmonella* Braenderup)

By using the turbidity meter, the cell density of 0.6-0.8 was measured. A 100µL cell suspension was transferred to a sterile microcentrifuge tube added with 2.6 µL of Proteinase K (20 mg/ml stock, Promega). Then, 120µL of molten 1% Seakem Gold Agarose and 20µL of Proteinase K were added to the cell suspension and mixed by gently tapping the tube. The mixed suspension was immediately transferred to a PFGE plug mould and settled for 10 min at room temperature.

After that, the plug was removed from the mould. It was lysed in a cell lysis buffer mix (CLB) which consisted of 2 mL cell lysis buffer 10 µL of Proteinase K. The plug that immersed in the CLB was incubated in a water bath at 54°C for overnight.

On the next day, the plus was first washed twice using sterile distilled water to clear the CLB on the plugs. Then, 1 × TE buffer was used to remove the cell debris thoroughly in the plugs. The plugs were washed in 1 × TE buffer for 6 times at room temperature

with agitation of 110 rpm. Plugs were later kept in a 2 mL 1 × TE buffer while waiting for restriction digestion.

Before restriction step, the plugs were sliced into the size of 1.5mm × 7mm. The sliced plugs were then placed into the microcentrifuge added with restriction buffer (1 × multicore buffer, sterile distilled water, 0.1 mg/mL BSA (Promega), 12U *Xba*I (for ***E. coli*** and ***Salmonella***) or 40U *Not*I (for ***V. cholerae***). The restriction of PFGE plugs was maintained at 37±2°C for overnight. The restriction buffer mix was then replaced 150µL of 0.5 × TBE buffer in each microcentrifuge to stop the restriction digestion step. Digested PFGE plugs could be kept at 4°C in the 1×TE buffer for a week before use.

Upon waiting for PFGE gel tank to be chilled to 14°C, the digested plug slices were settled on the PFGE comb for 8 minutes on the platform. A *Salmonella* Braenderup H9812 digested plug must be placed after every 4-6 samples. The molten Type I agarose (Sigma-Aldrich, USA) which was prepared earlier was poured onto the platform gently and solidified for 30 min. The solidified PFGE gel was then placed into the PFGE tank, and the condition was set according to Table 3.8.

PFGE for Gram-positive bacteria (*Staph. aureus*)

A cell density of 0.73-0.87 was adjusted in the cell suspension buffer and incubated at 37°C for 10 minutes. Four microliters of lysostaphin (Oxoid) and 15µL of lysozyme (Oxoid) were added to the cell suspension and incubated for another 30 min at 37°C. One microliter of Proteinase K (Promega) was added to the cell suspension. After incubation, 100µL of 1% molten SEAKEM Gold agarose was mixed well with the cell suspension and dispensed into the plug mould. These plugs were allowed to solidify at room temperature for 10 min.

Then, the plugs were lysed in CLB at 54°C for overnight. After lysis, the cell debris in the plugs was removed by washing steps. The plugs were washed twice with sterile distilled water and five times with 1 × TE buffer with agitation at 110 rpm. These plugs were stored at 4°C in 1 × TE buffer.

Before the digestion of PFGE plugs, the plugs were sliced into 1.5mm × 7mm. The plugs were digested in a mixture of 1 × Buffer J, 12 U BSA and 12 U *Sma*I at 37°C for overnight. On the next day, the digested plugs were embedded into Type I agarose and electrophoresed at the condition summarised in Table 3.8. The electrophoresed PFGE gel was staining in Gel Red (Biotium) for 30 min and viewed in a gel doc (Bio-Rad).

Table 3.8: The PFGE conditions for each bacterium.

Bacteria	Concentration of restriction enzyme used (U)	PFGE								
		State	Block	Run Time	Angle (°)	Voltage (V/cm)	Pulse time		Temperature (°C)	Running buffer
							Initial switch time	Final switch time		
E coli	12	Two state	-	23 h	120	6	2.16s	54.17s	14	0.5 × TBE
V. cholerae	40	Multistate	I	13h					14	0.5 × TBE
			state 1		60	6	2s	10s		
			state 2		-60	6	2s	10s		
			II	6h						
			state 1		60	6	20s	25s		
			state 2		-60	6	20s	25s		
Staph. aureus	12	Two state	-	22hr	120	6	5s	1min	14	0.5 × TBE

3.5 Research method- Food safety knowledge, attitudes, and practices assessment (*Part III*)

The ethical approval was obtained from the University of Malaya Medical Ethics Committee while the informed consents were obtained from the participants.

3.5.1 Questionnaire development

A set of questionnaire was developed adapted to the study by Osaili *et al.* (2013) and Pichler *et al*. (2014). It comprised of two parts: socio-demographics information and the questions on KAP. It is a bilingual questionnaire. The specimen of the questionnaire is attached as Appendix C.

The questionnaire was in dual languages (English and Malay language) and consisted of 97 items on demographic information (11 items), food safety knowledge (60 items), attitude (14 items) and self-reported practices (12 items). Food safety knowledge was assessed based on six constructs: (1) Personal hygiene, (2) cross-contamination prevention and sanitation, (3) food handling, (4) health problems that would affect food safety, (5) symptoms of foodborne diseases and (6) foodborne pathogens. The respondents were required to choose either "true" or "false" for each item on food safety knowledge and the score was given for each correct answer. The overall performance on food safety knowledge was converted to percentage by dividing the total score by a total number of items of food safety knowledge. While the food safety attitude and self-reported practices were assessed by four-level and five-level Likert scale questions, respectively. For items under attitude section, the lowest point (1 point) was given to "disagree" to the highest (4 points) for "agree"; while the self-reported practices were scored from the lowest (1 point) for "never" to the highest (5 points) for "always".

3.5.2 Data collection

This survey was conducted based on convenience sampling and depending on the willingness of the food handlers from December 2013 to August 2014. A face-to-face interview was given to the illiterate food handlers. A total of 67 questionnaires collected from the food handlers of contract or permanent status from twelve food premises within the campus from December 2013 to August 2014.

3.6 Research method- Data analysis

3.6.1 Cluster analysis

All REP-PCR and PFGE gel photos were exported into the Bionumerics version 7.1 Build 1.16911 (Applied Math, Kortrijk, Belgium). The banding patterns generated by REP-PCR and PFGE were analysed by using this software. Dice coefficient, F was used as the quantitative difference between the banding patterns, from 0 (dissimilar) to 1 (indistinguishable). The composite data were analysed by using 'average from experiments' while the unweighted pair group method with arithmetic mean was used as the clustering method. Discriminatory power (D) were obtained from the online software: http://insilico.ehu.es/mini_tools/discriminatory_power/?show=formula.

3.6.2 Statistical analysis

All statistical analyses included in this study were performed by using SPSS Statistics 22 (IBM, USA). The *p*-value was set at 0.05. Non-parametric Pearson Chi-Square, χ^2 was applied to determine the statistical significance of the prevalence of specific pathogens and the hygiene status of the RTE foods according to different food groups. Besides, the relationship between KAP results and the food handlers' socio-demographics was determined by using Mann-Whitney or Kruskal-Wallis tests. Independent samples T-test was applied in analyzing the significant differences between self-reported practices of food handling and the microbiological hygiene assessment results.

CHAPTER 4: RESULTS

4.1 Microbiological quality assessment on RTE foods, FCS, TCC and food handlers' hands

There was a total of 150 RTE foods, 59 FCS, 34 TCC and 85 hand swabs were collected randomly from the location mentioned. The raw data of the microbiological assessments are tabulated in Appendix D, E and F.

4.1.1 RTE foods

A total of 150 RTE foods which consisted of eight groups were collected randomly based on the availability within the campus and the vicinity of campus. Table 4.1 shows the overall microbiological quality of RTE foods collected. Among 150 RTE foods, 50% (n = 75) were of an unsatisfactory level of aerobic colony count (ACC), which means half of the samples harboured > 4 log CFU microorganisms. Whilst 24% of these RTE foods were contaminated with an unsatisfactory level of coliforms (> 2 log CFU). On the other hand, the *E. coli* and *Staph. aureus* contamination in RTE foods were low.

Statistical analysis showed that there were significant differences between the ACC ($p = 0.000$, df = 7, $\chi^2 = 36.556$) and coliform count ($p = 0.000$, df = 7, $\chi^2 = 36.556$) across the different food groups (Table 4.2). Food group D (uncooked RTE foods) had the highest percentage of unsatisfactory level in all the three hygiene parameters; whereas food group E (hot drink with milk) and F (hot drink without milk) scored the lowest level of unsatisfactory based on the ACC, coliforms and *E. coli* counts (Table 4.2). All the samples had a satisfactory level of *Staph. aureus*.

One-third (n = 50; 33.3%) of the RTE foods sold in the studied food premises were detected with *Salmonella* spp. from the range of 3 to 160 MPN/g (mL), from all the eight food groups, including hot beverages (Table 4.2). The samples detected with *Salmonella* spp. were further confirmed by DNA sequencing which is mentioned in section 4.1.4. However, the level of contamination was significantly higher in food group

D (n = 8; 61.5 %) with the MPN_{max} of 28 MPN/g ($p = 0.045$, df = 7, $\chi^2 = 14.372$) (Table 4.2). On the other hand, food group E (hot drinks with milk) had the lowest MPN range 3 to 7.4 MPN/g.

Out of 150 RTE samples tested, two samples from food group B (cooked food on display) and 1 sample from food group H (cold beverages without milk) were contaminated with 9.2 to 93 MPN/g (mL) of *V. cholerae*. On the other hand, *V. parahaemolyticus* was detected only in 2 samples (1.3%) from food group B and D with the range of 9.2 to 93 MPN/g (Table 4.2). Overall, the prevalence of *V. cholerae* and *V. parahaemolyticus* was low in this study.

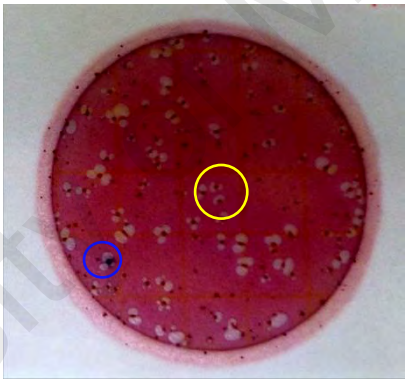


Figure 4.1 Representative photo of enumeration of *E. coli* on Petrifilm™ *E. coli*/coliform count plate.
The colony in blue with air bubbles (blue circle) was *E. coli* whilst the colonies in red with bubbles (yellow circle) were identified as coliforms.

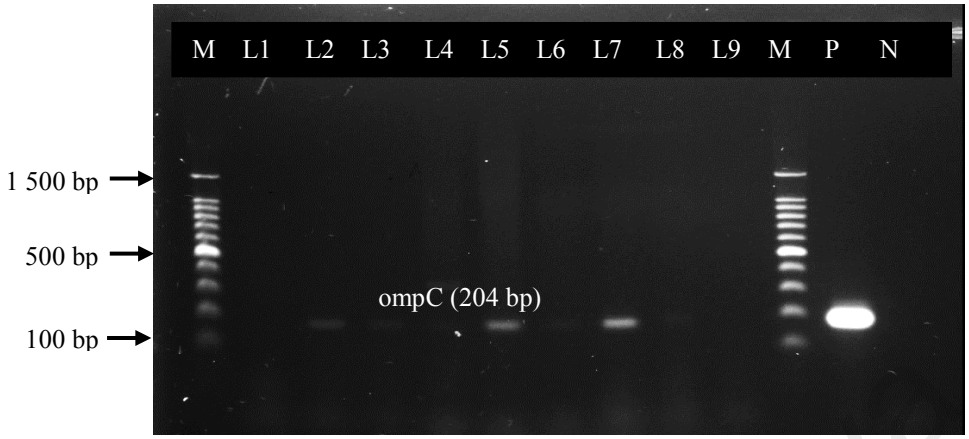


Figure 4.2: Representative gel photo of MPN-PCR enumeration of *Salmonella*.

Lane M is the molecular marker 100bp ladder; L1 to L9 shows the MPN-PCR samples from RTE foods (MO8); Lane P is a positive control and Lane N is the non-template control.

Table 4.1: Microbiological assessment results on RTE foods sampled from the food premises.

	RTE foods[c] (n=150)		
	Range	**n**	**%**
Hygiene indicators[a]			
Aerobic colony count	5 - > 10^5	75	50
Total coliforms	< 5 - > 10^5	114	76
E. coli	< 5 - > 10^3	145	97
Staph. aureus	< 5 - 5	150	100
Foodborne pathogens[b]			
Salmonella spp.	< 3 - 160	50	33
V. cholerae	< 3 - 93	3	2
V. parahaemolyticus	< 3 - 93	1	1

[a] The hygiene indicators were recorded for percentage of satisfactory and acceptable samples based on guidelines referred.
[b] The number of samples detected positive for the foodborne pathogens based on MPN-PCR method.
[c] The range is in a unit of CFU/g or MPN/g.

Table 4.2: The microbiological quality of RTE foods according to the respective food group.

Food group[c]	A n = 14		B n = 39		C n = 16		D n = 13		E n = 17		F n = 12		G n = 23		H n = 16		Total n = 150	*p*-value
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Hygiene indicators[a]																		
Aerobic colony count	9	64.3	27	69.2	15	93.8	12	92.3	2	11.8	1	8.3	6	26.1	3	18.8	75	0.000*
Total coliforms	2	14.3	8	20.5	10	62.5	9	69.2	1	5.9	1	8.3	4	17.4	1	6.3	36	0.000*
E. coli	1	7.1	2	5.1	0	0	2	15.4	0	0	0	0	0	0	0	0	5	0.202
Staph. aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA
Foodborne pathogens[b]																		
Salmonella spp.	6	42.9	9	23.1	8	50.0	8	61.5	4	23.5	3	25.0	10	43.5	2	12.5	50	0.045*
V. cholerae	0	0	2	5.1	0	0	0	0	0	0	0	0	0	0	1	6.3	3	0.616
V. parahaemolyticus	0	0	1	2.6	0	0	1	7.7	0	0	0	0	0	0	0	0	2	0.567

*Statistically significant, $p < 0.05$ by Pearson Chi-Square test. NA: Not applicable because of *Staph. aureus* assessment is a constant.
n number of samples
[a] The hygiene indicators were recorded for a percentage of unsatisfactory samples based on guidelines referred (Table 3.2).
[b] The samples that were detected positive for foodborne pathogens by MPN-PCR method.
[c] A, Cooked food for immediate consumption; B, Fully cooked food kept warm on a heater or display; C, Combination of cooked and uncooked RTE foods; D, Uncooked food that ready for consumption; E, Hot drinks with milk; F, Hot drinks without milk; G, Cold drinks with milk; H, Cold drinks without milk.

4.1.2 Food contact surfaces and table cleaning cloths

Approximately 95% of FCS constantly yielded more than 1.3 log CFU/cm^2 of aerobic colony count, 63% FCS samples harboured > 1.0 log CFU/cm^2 of coliforms while the satisfactory levels of *E. coli* and *Staph. aureus* both were higher than 90% (Table 4.3). Only 12% (n = 4) of the TCC samples were of satisfactory level of ACC. The prevalence of *Salmonella* in FCS and TCC were 37% (MPN = 3 to > 1 100 MPN/100 cm^2) and 62% (MPN = 3.6 to 230 MPN/cloth), respectively. Forty-one percent (n = 14) of the TCC were contaminated with *V. cholerae* with the MPN of 3.6 to > 1 100 MPN/ cloth. More than half of the FCS and TCC were of the unsatisfactory level of ACC.

Table 4.3: Microbiological assessment results on FCS and TCC sampled from the food premises located within the campus.

	FCS[c] (n = 59)			TCC[d] (n = 34)		
	Range	n	%	Range	n	%
Hygiene indicators[a]						
Aerobic colony count	< 5 - > 10^5	3	5	< 5 - > 10^5	4	12
Total coliforms	< 5 - > 10^5	16	27	< 5 - > 10^5	10	29
E. coli	< 5 - > 10^3	54	92	< 5 - > 10^3	29	85
Staph. aureus	< 5 - 620	53	90	< 5 - > 10^3	28	82
Foodborne pathogens[b]						
Salmonella spp.	< 3 - 120	22	37	< 3 - > 1100	21	62
V. cholerae	< 3 - 230	4	7	< 3 - > 1100	14	41
V. parahaemolyticus	< 3	0	0	< 3 – 220	2	6

FCS, Food contact surfaces; TCC, Table cleaning cloth
[a] The hygiene indicators were recorded for percentage of satisfactory and acceptable samples based on guidelines referred.
[b] The number of samples detected positive for the foodborne pathogens based on MPN-PCR method.
[c] The range is in a unit of CFU/cm^2 or MPN/cm^2.
[d] The range is in a unit of CFU/cloth or MPN/cloth.

4.1.3 Validation of MPN-PCR

In order to eliminate the false positive in MPN-PCR, the PCR products (samples detected with *Salmonella* and *V. cholerae* DNA) were sent for sequencing. The sequencing data obtained from the commercial laboratory were blasted against the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). All sequenced data were listed in Table 4.4.

Table 4.4: The results of samples sequenced to validate the MPN-PCR results.

Sample	Source	Description	Attachment
TC1-2	TCC	*S. enterica* Typhimurium	Appendix G
IC18-4	Food	*S. enterica* Enteritidis	
CB1-5	Cutting board	*S. enterica* Typhimurium	
FH66-3	Food handler	*S. enterica* Enteritidis	
TC10-1	TCC	*V. cholerae*	

4.1.4 Food handlers

All data collected were tabulated in Appendix D. Based on the microbial assessment results, 64.7% (n = 55) of the food handlers had an unsatisfactory level of aerobic colony count (≥ 1.3 log CFU/cm^2; Table 4.4). One-third (35.3%, n – 30) of the food handlers had ≥ 1.3 log CFU/cm^2 of total coliforms on their hands. Overall, the *E. coli* and *Staph. aureus* counts were maintained in a high compliance rate. Surprisingly, the prevalence of *Salmonella* was extremely high (48%, n = 41) with the microbial load ranged from < 3 to 150 MPN/person.

Table 4.5: Microbiological assessment results on food handlers' hands sampled from the food premises located within the campus.

Microbial indicator	Status	Number	Percentage (%)	Min[a]	Max[a]
Aerobic colony count[c]					
	Fail	55	64.7	1.36	> 1.46
	Pass	30	35.5	< 0.1	1.27
Coliform[d]					
	Fail	30	35.3	1.3	> 3.18
	Pass	55	64.7	< 0.1	2.64
***E. coli*[e]**					
	Fail	2	2.4	1.08	1.11
	Pass	83	97.6	n.d.	2.45
***Staph. aureus*[f]**					
	Fail	3	3.5	1.04	> 1.46
	Pass	82	96.5	n.d.	0.70
Foodborne pathogens	**Presence**	**Number**	**Percentage (%)**	**MPN_{min} per person[b]**	**MPN_{max} per person[b]**
Salmonella					
	Detected	41	48.2	3.0	150
	Not detected	44	51.8	< 3.0	n.a.
V. cholerae					
	Detected	2	2.4	3.6	n.a.
	Not detected	83	97.6	< 3.0	n.a.
V. parahaemolyticus					
	Detected	1	1.2	23.0	n.a.
	Not detected	84	98.8	< 3.0	n.a.

n.d.: not detected or below detection limit; n.a.: not applicable

[a] The minimum (Min) and maximum (Max) CFU/ cm^2.

[b] The minimum (MPN_{min})and maximum (MPN_{max}) MPN value per person (a pair of hands)

[c]Aerobic count threshold based on Tan *et al.,* 2013 and Sneed *et al.,* 2004, which is ≥ 1.3 log CFU/cm^2.

[d] Coliform count threshold based on Tan *et al*., 2013 and Sneed *et al*., 2004, which is ≥ 1.3 log CFU/cm^2.

[e]*E. coli* count threshold based on Tan *et al*., 2013 and Sneed *et al*., 2004, which is ≥ 1.0 log CFU/cm^2.

[f] *Staph. aureus* count threshold based on Tan *et al*., 2013 and Sneed *et al*., 2004, which is ≥ 1.0 log CFU/cm^2.

4.2 Bacterial isolation and identification

All presumptive isolates were identified by biochemical tests (Table 4.6) and confirmed by PCR (Figure 4.4). There were 130 *E. coli*, 81 *Staph. aureus* and 26 *V. cholerae* isolated from RTE foods, FCS, TCC and food handlers' hands. Representative isolates were sent for 16S rRNA sequencing to validate the confirmed isolates. The sequencing results were attached in Appendix I while the list of bacteria isolated was tabulated in Appendix H. Table 4.7 shows the attribution of the bacterial isolates from different sources.

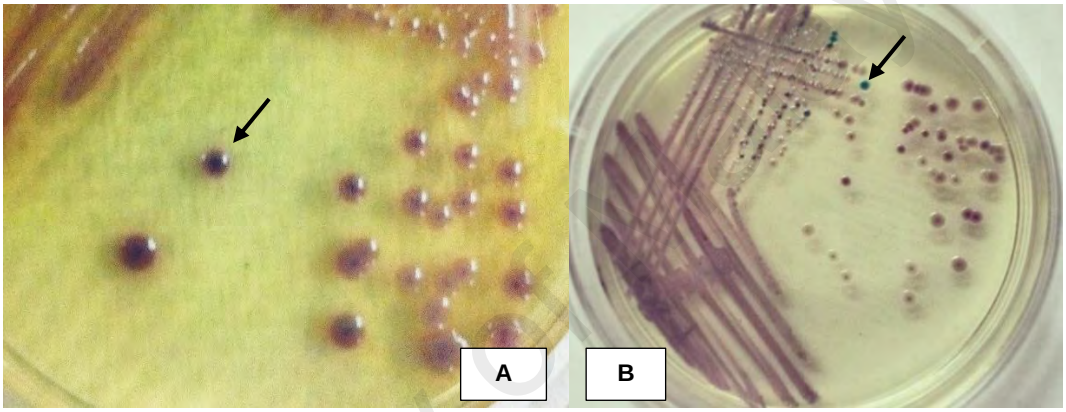


Figure 4.3: Representative photos of isolation of *E. coli* (A) and *V. cholerae* (B) on CHROMagar Orientation and CHROMagar Vibrio, respectively. Arrow pointed at the presumptive *V. cholerae*.

Although there were presumptive *Salmonella* isolates grown on CHROMagar *Salmonella*. However, none of presumptive *Salmonella* isolates was confirmed as *Salmonella* in both biochemical test and PCR. Therefore, the detected *Salmonella* DNA could either be the DNA from dead cells of *Salmonella* spp. or the viable but non-culturable cells.

Table 4.6: Biochemical properties of each bacteria.

Target bacteria	Colour on differential/ selective medium	String test	Oxidase test
E. coli	Mauve	Positive	Negative
Staph. aureus	Yellow	Negative	Negative
V. cholerae	Turquoise	Positive	Positive

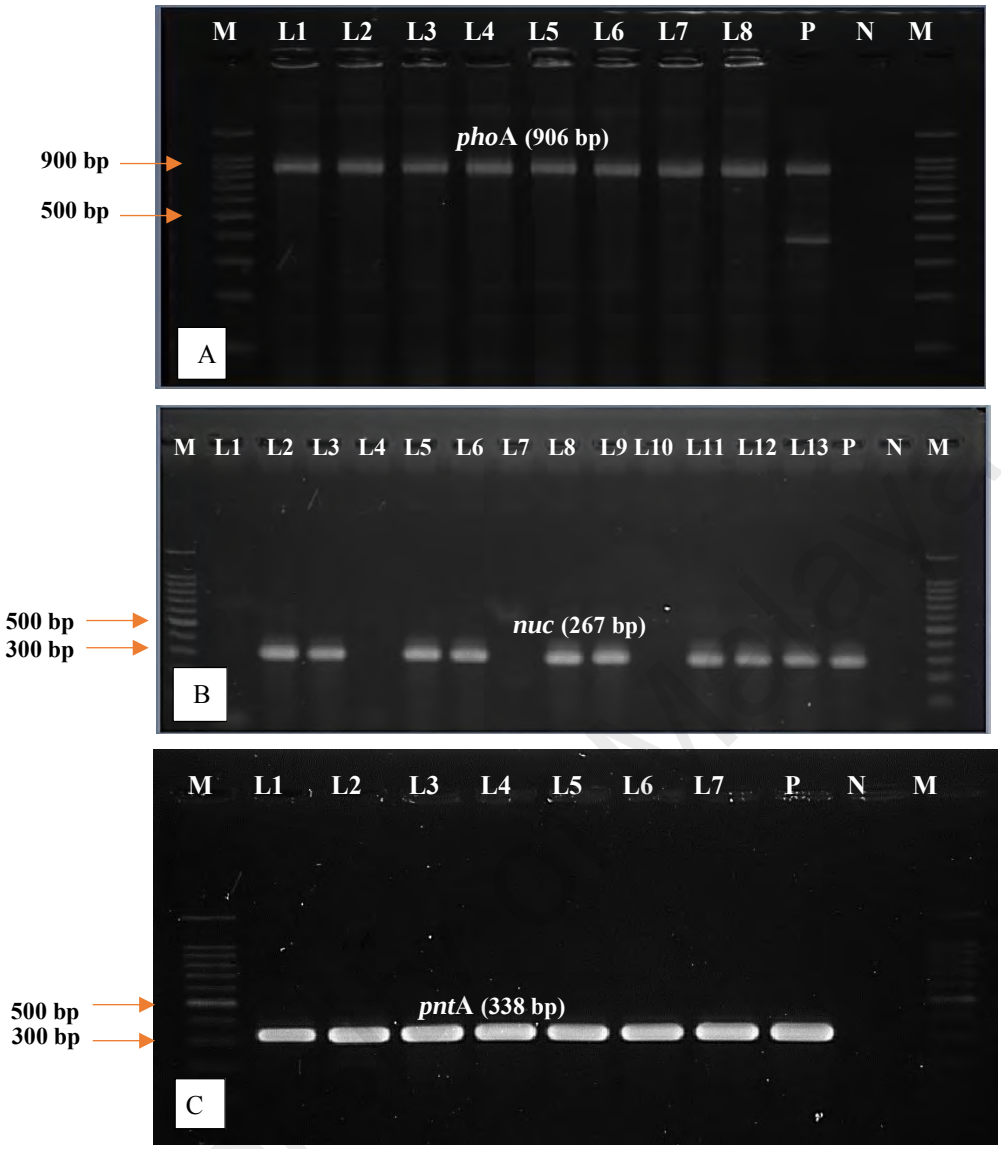


Figure 4.4: Representative gel photos of species-specific PCR identification (A- *E. coli*, B- *Staph. aureus* and C- *V. cholerae*) for bacteria isolated.

Table 4.7: Bacteria isolated from different sources.

Bacteria	Total	RTE food		FCS		TCC		Food handler	
		n	%	n	%	n	%	n	%
E. coli	130	50	38.5	10	7.7	39	30	31	23.9
Staph. aureus	81	4	5	9	11	12	15	56	69
V. cholerae	26	0	0	0	0	26	100	0	0

Note: Bacterial species had been confirmed by PCR identification.

4.2.1 Antibiograms of *E. coli*

Thirty-six resistotypes were observed among the *E. coli* isolates (Figure 4.5). Two resistotypes: TE^R (n = 77, 59.2%) and E^R (n = 128, 98.5%) were dominant among the resistotypes. All *E. coli* were susceptible to carbapenems (Imipenem and Meropenem) while only one isolate was resistant to Cephems (only Cefuroxime). Approximately two-third (n = 88) of the *E. coli* were resistant to more than one antimicrobial agent. It was also noticed that the co-resistance of tetracycline and erythromycin was the highest, 98.7%, followed by both ampicillin and trimethoprim-sulfamethoxazole, 45.5%.

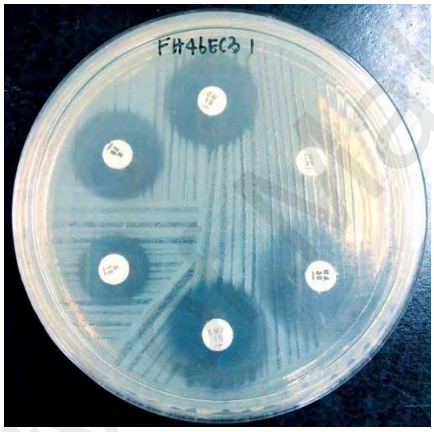


Figure 4.5: An example of the antimicrobial susceptibility testing.

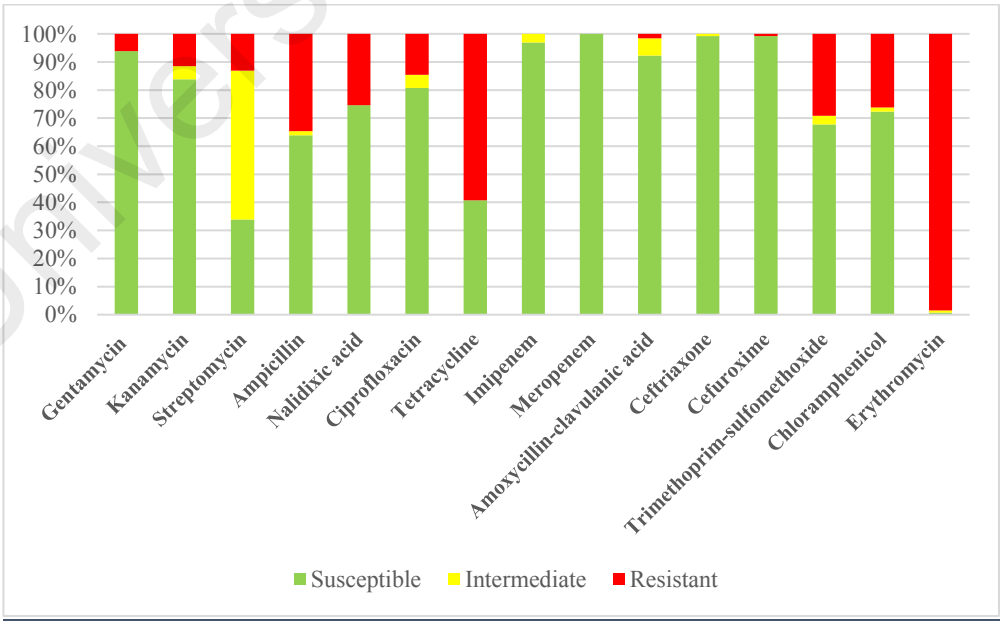
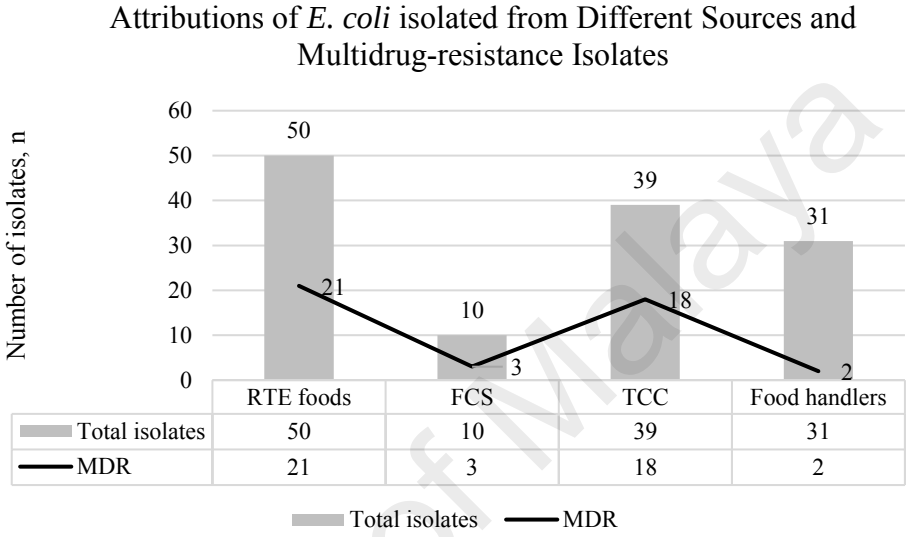


Figure 4.6: Chart of *E. coli* antibiograms

Out of 130 isolates, 33.8% (n = 44) were multidrug resistance (MDR) *E. coli*. Forty isolates (30.8%) were resistant to ≥ 6 antibiotics. The percentage of multidrug resistance was found higher in *E. coli* isolated from TCC (n = 18, 46.2%) and RTE foods (n = 21, 42.0%). Interestingly, an isolate from cutting board was resistant to 11 types of antibiotics or 9 groups of antibiotics.



	RTE foods	FCS	TCC	Food handlers
Total isolates	50	10	39	31
MDR	21	3	18	2

Figure 4.7: Attributions *of E. coli* isolated from different sources and multidrug-resistance isolates

4.2.2 Virulence profiles of *E. coli*

Six virulence genes were tested. None of the isolates was detected positive in the virulence genes. These *E coli* isolates do not carry any virulence genes that were tested.

4.2.3 REP profiles of *E. coli*

All isolates were typeable by REP-PCR and reproducible banding patterns were obtained. The DNA fragments varied from 250 bp to 1 500 bp (Figure 4.8). *E. coli* isolated were genetically diverse as 76 unique profiles ranging from 3 to 14 bands. The discriminatory power was 0.9875 while the Simpson's index was 0.9870.

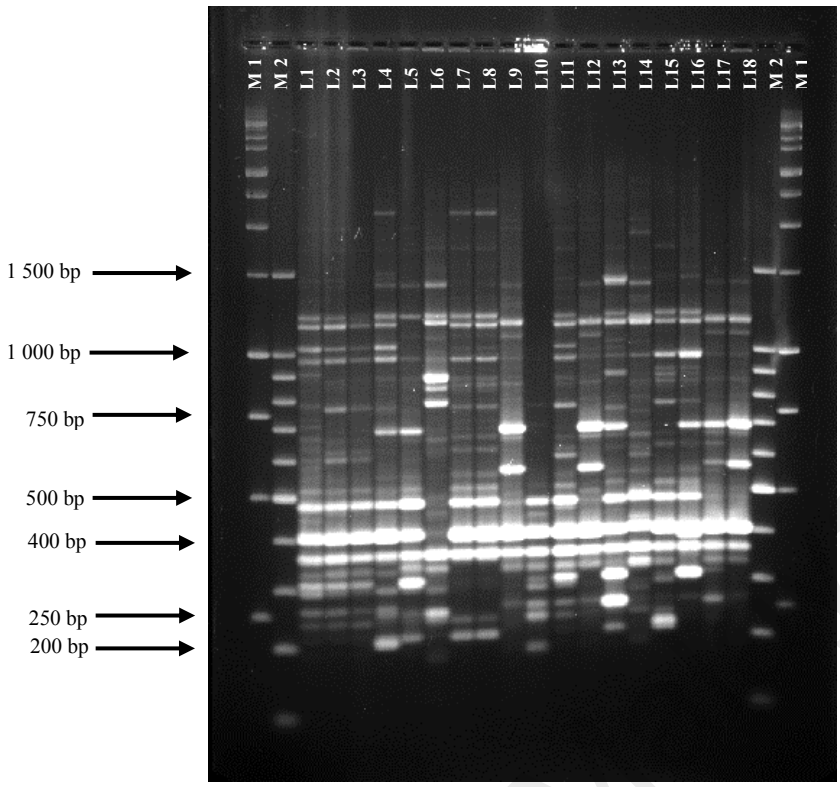


Figure 4.8: Representative gel photo of REP-PCR gel of *E. coli.*
M1, 1 000 bp molecular ladder; M2, 100 bp molecular ladder; L1, FH63EC1; L2, TC27EC1; L3, TC27EC3; L4, TC11EC2; L5, CB1EC1; L6, V137EC4; L7, TC12EC2; L8, TC12EC4; L9, FH46EC1; L10, TC13EC1; L11, CB36EC3; L12, FH44EC6; L13, FH50EC1; L14, CB38EC1; L15, FH65EC2; L16, CB35EC2; L17, FH63EC3; and L18, FH46EC5.

To determine the clonal relationship of isolates (excluding duplicates), only those isolated from different sources and of different banding patterns were selected for cluster analysis using the UPGMA algorithm (Figure 4.9). Group A, C, and D consist of 4, 11 and two strains, respectively which shared close similarity among them. Members of each cluster were isolated from the same food premise. Hence, it is not surprising that these isolates are closely related. The same resistotypes were observed within the Group B, C, and D, which was mainly due to the same sampling sites.

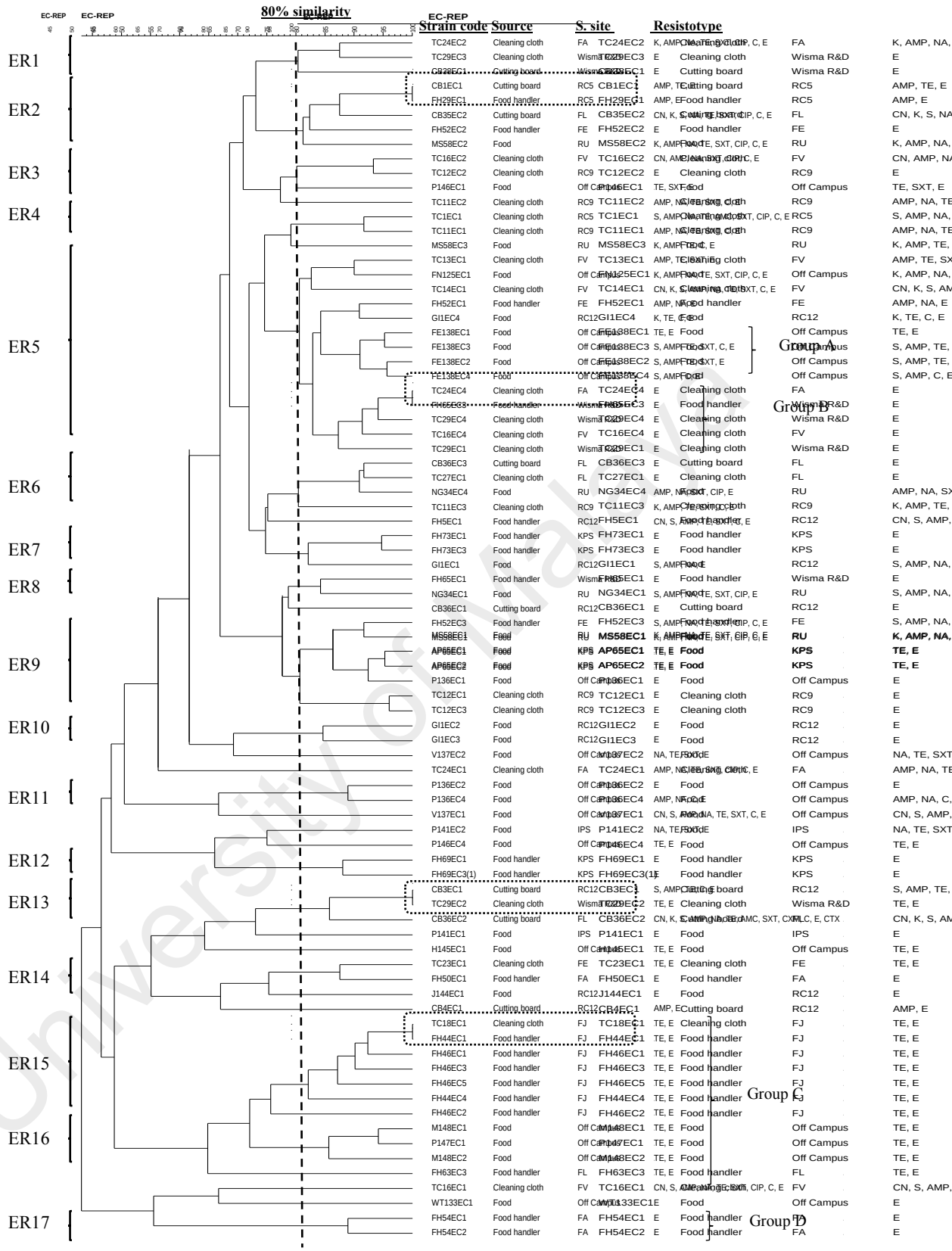


Figure 4.9: Dendrogram generated from the cluster analysis of *E. coli* subtyping. The dashed boxes show the *E. coli* that are 100% similar to each other.

Eighty-two *E. coli* strains were sub-grouped into 17 clusters at 80% similarity. RE5 was the biggest cluster which contained 14 strains. This cluster comprised of *E. coli* isolated from food handlers, TCC and foods. Similar observation was noted for strains within clusters ER2, ER 3, ER5, ER6, ER7, ER8 and ER9, in which the *E. coli* isolated from TCC/ cutting boards/ food handlers were closely related to *E. coli* isolated from RTE foods.

It was noted that certain *E. coli* from different sources, particularly from a food handler and TCC; food handler and cutting board; cutting board and TCC had indistinguishable REP-PCR profile shown in the dotted boxes illustrated in Figure 4.9, indicating possibility of a cross-contamination event in the food premises.

4.2.4 PFGE profiles of *E. coli*

Nine isolates were untypeable by PFGE in spite of repeated analysis. PFGE produced reproducible banding patterns which comprised of 10 to 20 bands with the DNA size varied from 20.5 bp to 452.7 bp (Figure 4.10).

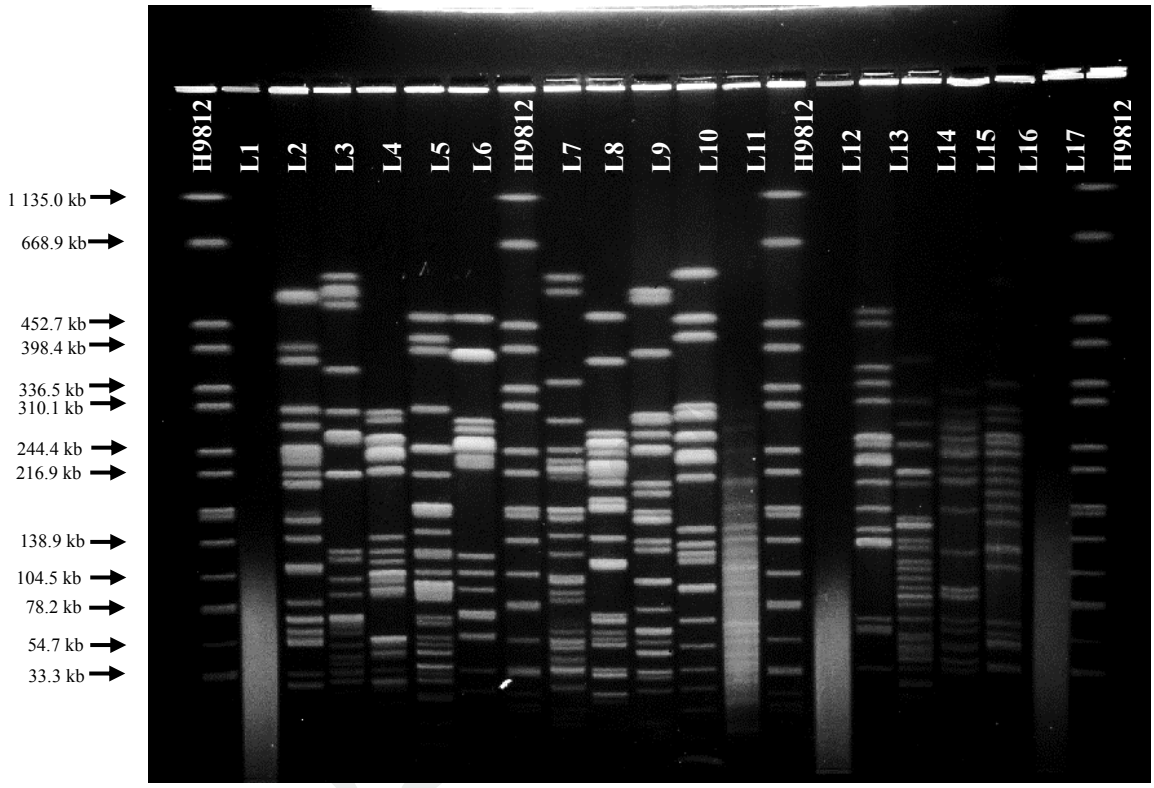
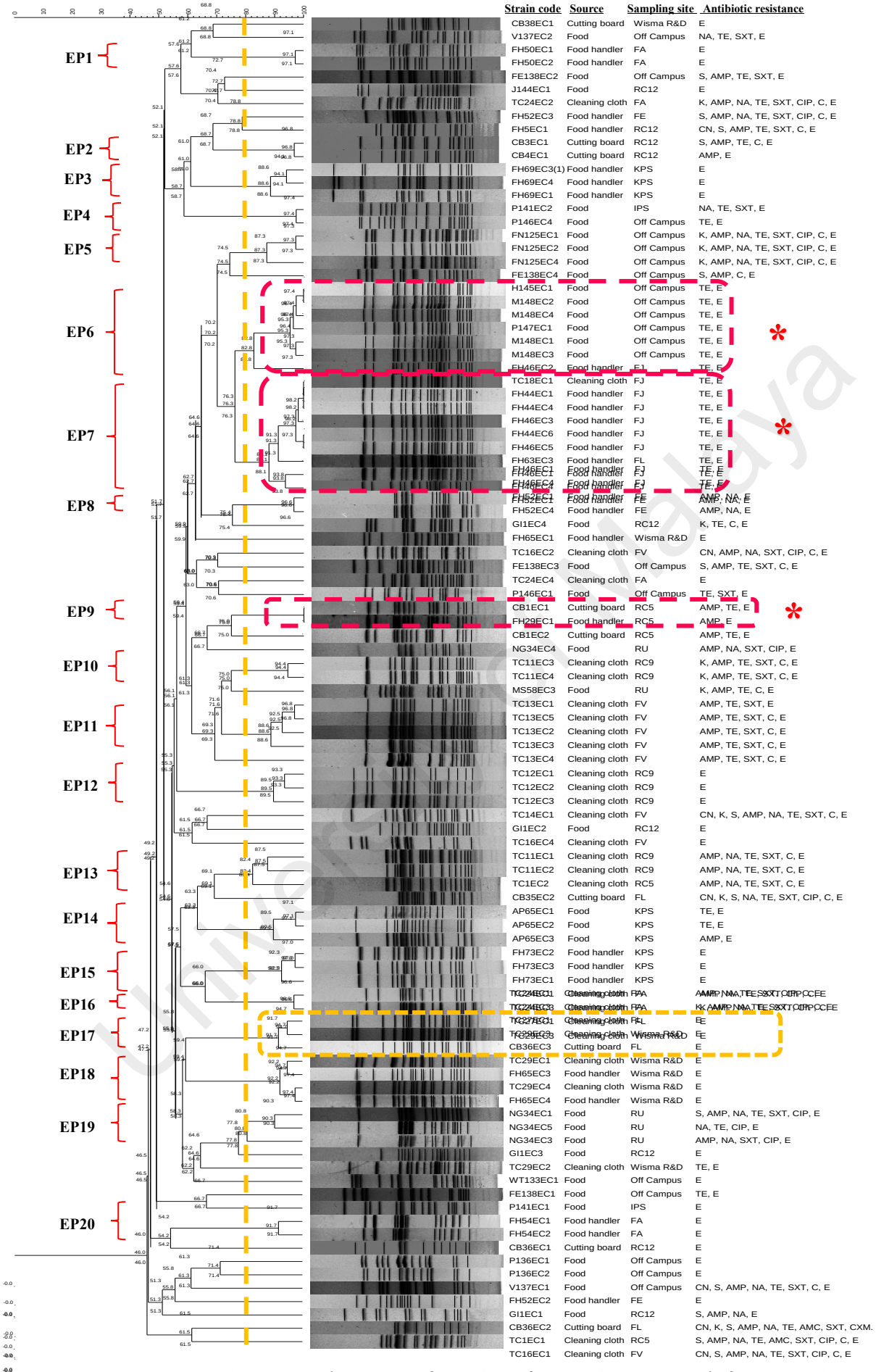


Figure 4.10: Representative PFGE gel photo of *E. coli*.
L1, TC23EC3; L2, FH73EC2; L3, WT133EC2; L4, FH52EC4; L5, FN125EC1; L6, FH54EC1; L7, TC27EC1; L8, TC24EC3; L9, TC24EC4; L10, TC12EC4; L11, M148EC3; L12, MS58EC2; L13, P136EC2; L14, GI1EC2; L15, FH52EC2; L16, AP65EC2 & L17, TC23EC4 were the *E. coli* isolated while H9812 was the universal reference standard, *S*. Braenderup.

There were 95 unique profiles generated from PFGE (Figure 4.11) with the *D* value= 0.9937. Indistinguishable pulsotypes were observed on the *E. coli* isolates in which were isolated from different sources and sampling sites (Figure 4.11, indicated with red asterisk '*' and red box). The indistinguishable strains from cutting board and food handlers were observed in both REP and PFGE fingerprinting methods. Clusters EP7 was the cluster that contained the highest number of strains; all strains were isolated

from the same sampling site. On the other hand, Cluster EP17 (orange box) consisted three strains isolated from different sampling site and samples (TCC and Cutting board) but having high similarity.

E. coli strains that were subtyped into the same cluster had similar antimicrobial resistance profile. This scenario was observed in most of the clusters, except for EP2, EP4, EP16, and EP19.



Strain code	Source	Sampling site	Antibiotic resistance
CB38EC1	Cutting board	Wisma R&D	E
V137EC2	Food	Off Campus	NA, TE, SXT, E
FH50EC1	Food handler	FA	E
FH50EC2	Food handler	FA	E
FE138EC2	Food	Off Campus	S, AMP, TE, SXT, E
J144EC1	Food	RC12	E
TC24EC2	Cleaning cloth	FA	K, AMP, NA, TE, SXT, CIP, C, E
FH52EC3	Food handler	FE	S, AMP, NA, TE, SXT, CIP, C, E
FH5EC1	Food handler	RC12	CN, S, AMP, TE, SXT, C, E
CB3EC1	Cutting board	RC12	S, AMP, TE, C, E
CB4EC1	Cutting board	RC12	AMP, E
FH69EC3(1)	Food handler	KPS	E
FH69EC4	Food handler	KPS	E
FH69EC1	Food handler	KPS	E
P141EC2	Food	IPS	NA, TE, SXT, E
P146EC4	Food	Off Campus	TE, E
FN125EC1	Food	Off Campus	K, AMP, NA, TE, SXT, CIP, C, E
FN125EC2	Food	Off Campus	K, AMP, NA, TE, SXT, CIP, C, E
FN125EC4	Food	Off Campus	K, AMP, NA, TE, SXT, CIP, C, E
FE138EC4	Food	Off Campus	S, AMP, C, E
H145EC1	Food	Off Campus	TE, E
M148EC2	Food	Off Campus	TE, E
M148EC4	Food	Off Campus	TE, E
P147EC1	Food	Off Campus	TE, E
M148EC1	Food	Off Campus	TE, E
M148EC3	Food	Off Campus	TE, E
FH46EC2	Food handler	FJ	TE, E
TC18EC1	Cleaning cloth	FJ	TE, E
FH44EC1	Food handler	FJ	TE, E
FH44EC4	Food handler	FJ	TE, E
FH46EC3	Food handler	FJ	TE, E
FH44EC6	Food handler	FJ	TE, E
FH46EC5	Food handler	FJ	TE, E
FH63EC3	Food handler	FL	TE, E
FH46EC1	Food handler	FJ	TE, E
FH46EC4	Food handler	FJ	TE, E
FH52EC1	Food handler	FE	AMP, NA, E
FH52EC4	Food handler	FE	AMP, NA, E
GI1EC4	Food	RC12	K, TE, C, E
FH65EC1	Food handler	Wisma R&D	E
TC16EC2	Cleaning cloth	FV	CN, AMP, NA, SXT, CIP, C, E
FE138EC3	Food	Off Campus	S, AMP, TE, SXT, C, E
TC24EC4	Cleaning cloth	FA	E
P146EC1	Food	Off Campus	TE, SXT, E
CB1EC1	Cutting board	RC5	AMP, TE, E
FH29EC1	Food handler	RC5	AMP, E
CB1EC2	Cutting board	RC5	AMP, TE, E
NG34EC4	Food	RU	AMP, NA, SXT, CIP, E
TC11EC3	Cleaning cloth	RC9	K, AMP, TE, SXT, C, E
TC11EC4	Cleaning cloth	RC9	K, AMP, TE, SXT, C, E
MS58EC3	Food	RU	K, AMP, TE, C, E
TC13EC1	Cleaning cloth	FV	AMP, TE, SXT, E
TC13EC5	Cleaning cloth	FV	AMP, TE, SXT, C, E
TC13EC2	Cleaning cloth	FV	AMP, TE, SXT, C, E
TC13EC3	Cleaning cloth	FV	AMP, TE, SXT, C, E
TC13EC4	Cleaning cloth	FV	AMP, TE, SXT, C, E
TC12EC1	Cleaning cloth	RC9	E
TC12EC2	Cleaning cloth	RC9	E
TC12EC3	Cleaning cloth	RC9	E
TC14EC1	Cleaning cloth	FV	CN, K, S, AMP, NA, TE, SXT, C, E
GI1EC2	Food	RC12	E
TC16EC4	Cleaning cloth	FV	E
TC11EC1	Cleaning cloth	RC9	AMP, NA, TE, SXT, C, E
TC11EC2	Cleaning cloth	RC9	AMP, NA, TE, SXT, C, E
TC1EC2	Cleaning cloth	RC5	AMP, NA, TE, SXT, C, E
CB35EC2	Cutting board	FL	CN, K, S, NA, TE, SXT, CIP, C, E
AP65EC1	Food	KPS	TE, E
AP65EC2	Food	KPS	TE, E
AP65EC3	Food	KPS	AMP, E
FH73EC2	Food handler	KPS	E
FH73EC3	Food handler	KPS	E
FH73EC1	Food handler	KPS	E
TC24EC1	Cleaning cloth	FA	AMP, NA, TE, SXT, CIP, C, E
TC24EC3	Cleaning cloth	FA	K, AMP, NA, TE, SXT, CIP, C, E
TC27EC1	Cleaning cloth	FL	E
TC29EC3	Cleaning cloth	Wisma R&D	E
CB36EC3	Cutting board	FL	E
TC29EC1	Cleaning cloth	Wisma R&D	E
FH65EC3	Food handler	Wisma R&D	E
TC29EC4	Cleaning cloth	Wisma R&D	E
FH65EC4	Food handler	Wisma R&D	E
NG34EC1	Food	RU	S, AMP, NA, TE, SXT, CIP, E
NG34EC5	Food	RU	NA, TE, CIP, E
NG34EC3	Food	RU	AMP, NA, SXT, CIP, E
GI1EC3	Food	RC12	E
TC29EC2	Cleaning cloth	Wisma R&D	TE, E
WT133EC1	Food	Off Campus	E
FE138EC1	Food	Off Campus	TE, E
P141EC1	Food	IPS	E
FH54EC1	Food handler	FA	E
FH54EC2	Food handler	FA	E
CB36EC1	Cutting board	RC12	E
P136EC1	Food	Off Campus	E
P136EC2	Food	Off Campus	E
V137EC1	Food	Off Campus	CN, S, AMP, NA, TE, SXT, C, E
FH52EC2	Food handler	FE	E
GI1EC1	Food	RC12	S, AMP, NA, E
CB36EC2	Cutting board	FL	CN, K, S, AMP, NA, TE, AMC, SXT, CXM.
TC1EC1	Cleaning cloth	RC5	S, AMP, NA, TE, AMC, SXT, CIP, C, E
TC16EC1	Cleaning cloth	FV	CN, S, AMP, NA, TE, SXT, CIP, C, E

Figure 4.11: Dendrogram of PFGE of *E. coli* generated from UPGMA clustering method using dice coefficient analysis.

Ten resistotypes were observed among the 81 *Staph. aureus* isolates, in which P^R (n = 31, 38.3%) and TE^R (n = 9, 11.1%) were most common. The majority of the strains were pan-susceptible to the antibiotics tested. It was noted that three strains isolated from food handlers' hands were multidrug-resistant. They were resistant to 4 to 7 drug classes.

Table 4.8: Antibiograms of *Staph aureus*

Antibiotics (µg)	No. of resistance strain (n)	Percentage of resistance (%)
Penicillin G (10 U)	31	38.3
Teicoplanin (30)	2	2.5
Gentamycin (10)	0	0
Kanamycin (30)	8	9.9
Erythromycin (15)	6	7.4
Ciprofloxacin (5)	5	6.2
Tetracycline (30)	9	11.1
Clindamycin (2)	2	2.5
Trimethoprim-sulfomethoxazole (25)	0	0
Chloramphenicol (30)	2	2.5
Rifampicin (5)	2	2.5
Linezolid (30)	3	37

4.2.6 Virulence profiles of *Staph. aureus*

Among the strains, 13 out of 21 most reported food poisoning-causing virulence genes (including enterotoxin genes) were detected (Table 4.9). The representative strains sent for sequencing and analysed using NCBI showed high similarity (97% to 100% homology) to the reference strains in the database (Appendix J). Figure 4.12 shows the representative gel photos of PCR detection of virulence genes in *Staph. aureus*.

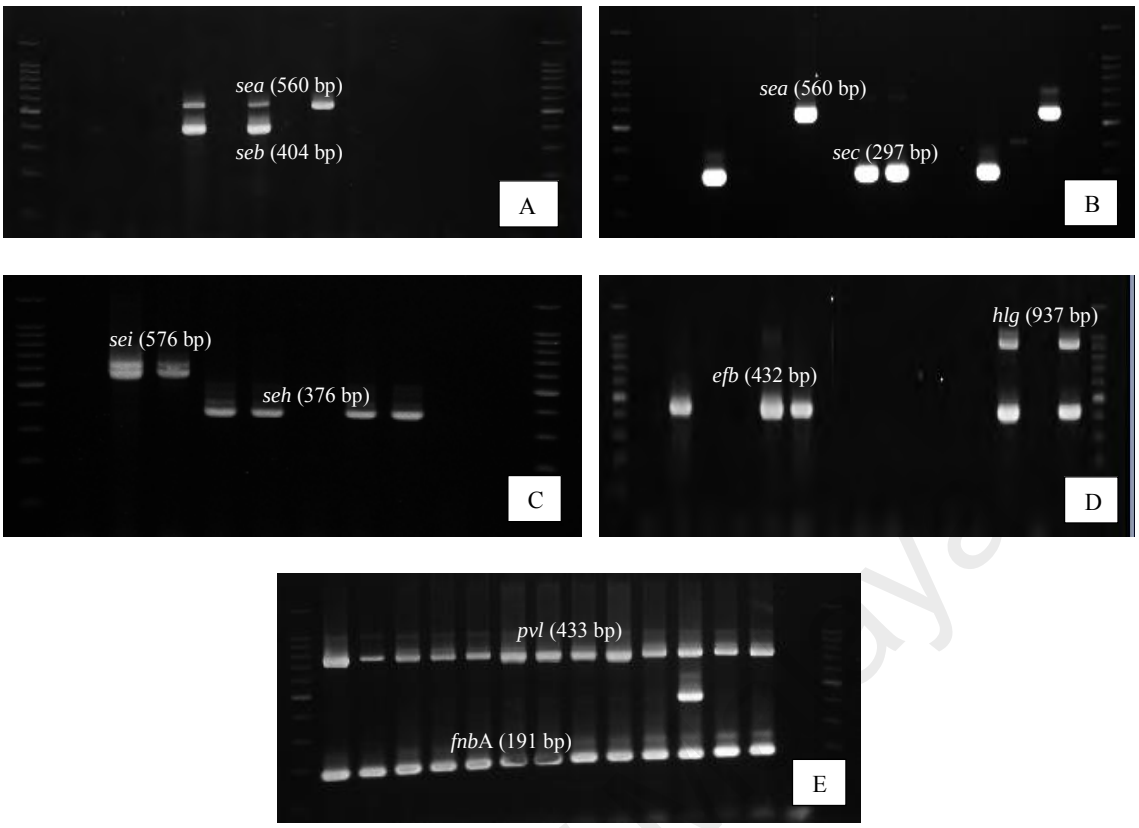


Figure 4.12: A series of representative gel photos for PCR detection of virulence genes in *Staph. aureus*.
Gel photo (A) indicates the presence of *sea* and *seb*; (B) indicates the presence of *sea* and *sec*; (C) indicates the presence of *sei*, and *seh*; (D) indicates the presence of *efb* and *hlg*; and (E) *ica*, *pvl*, and *fnb*A in the *Staph. aureus* isolates.

Strong emetic enterotoxins: *sea* to *seh* were detected among the strains, except for *see*. Most of them were isolated from food handlers' hands. Enterotoxin *sec* present in 16% of the strains, 9 out of 13 strains were isolated from the food handlers' hands.

The virulence gene, *fnb*A which encodes for fibronectin adhesin was dominant among the strains, followed by *efb* (n = 36, 44%) and *hlg* (n = 32, 40%) which inhibits platelet aggregation and promotes survival in human blood, respectively. There were only 3 strains that did not carry any virulence gene. Majority of the strains (n = 63, 80.8%) harboured 1 to 4 virulence genes. There were 15 strains (19.2%) harboured ≥ 5 virulence genes.

Table 4.9: Prevalence of virulence genes in *Staph. aureus*

Virulence gene	Number of strain detected positive	Percentage
sea	11	14
seb	6	7.4
sec	13	16
sed	2	2.5
see	0	0
seg	4	5
seh	12	15
sei	10	12
sej	1	1
efb	36	44
hlg	32	40
cna	0	0
eta	0	0
etb	0	0
etd	0	0
tst	0	0
ica	23	28
***fnb*A**	78	96
***fnb*B**	0	0
pvl	1	1
***sdr*E**	0	0

Table 4.10: Virulence profiles of *Staph. aureus*

Virulence gene	**No of strain harbour the listed gene**	**Virulence gene**	**No of strain harbour the listed gene**
*efb, fnb*A	1	*sec, ica ,fnb*A	2
*efb, hlg, fnb*A	3	*sec, ica, fnb*A	1
*efb, hlg, ica, fnb*A	4	*sec, seg, sei, efb, fnb*A	1
efb, hlg, ica, fnbA, pvl	1	*sec, sei, hlg, fnb*A	1
*efb, ica, fnb*A	1	*sec,seg, sei, efb, hlg, ica, fnb*A	1
*fnb*A	25	*sed, efb, hlg, fnb*A	1
*ica, fnb*A	4	*seg, efb, fnb*A	1
*sea, efb, hlg, fnb*A	1	*seg, sei, efb, fnb*A	1
*sea, efb, hlg, ica, fnb*A	1	*seh, efb, fnb*A	1
*sea, seb, seh, efb, hlg, fnb*A	2	*seh, efb, hlg, fnb*A	1
*sea, seb, seh, efb, hlg, fnb*A	2	*seh, efb, hlg, ica, fnb*A	1
*sea, seb, seh, efb, hlg, ica, fnb*A	1	*seh, efb, hlg, ica, fnb*A	1
*sea, sed, efb, hlg, ica, fnb*A	1	*seh, fnb*A	1
*sea, seh, efb, hlg, fnb*A	1	*seh, ica ,fnb*A	1
*sea, seh, sej, efb, hlg, ica, fnb*A	1	*sei, ,fnb*A	1
*sea,fnb*A	1	*sei, efb, hlg, fnb*A	1
*seb, fnb*A	1	*sei, hlg, fnb*A	1
*sec, efb, hlg, fnb*A	4	*sej, efb, hlg, fnb*A	1
*sec, efb, hlg, ica ,fnb*A	1	*sej, fnb*A	1
*sec, fnb*A	2		

4.2.7 REP profiles of *Staph. aureus*

The typeabliliy of REP-PCR in *Staph. aureus* strains achieved 100%. REP-PCR produce reproducible banding patterns ranging from 3 bands to 14 bands. Only DNA ranging from 250 bp to 1500bp were scored in the analysis.

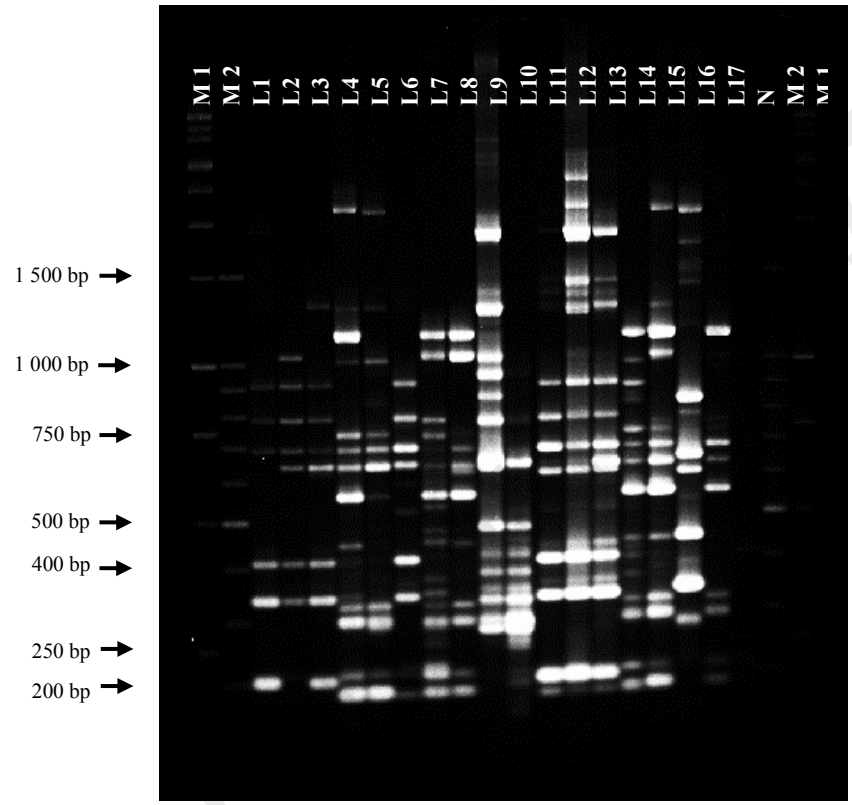
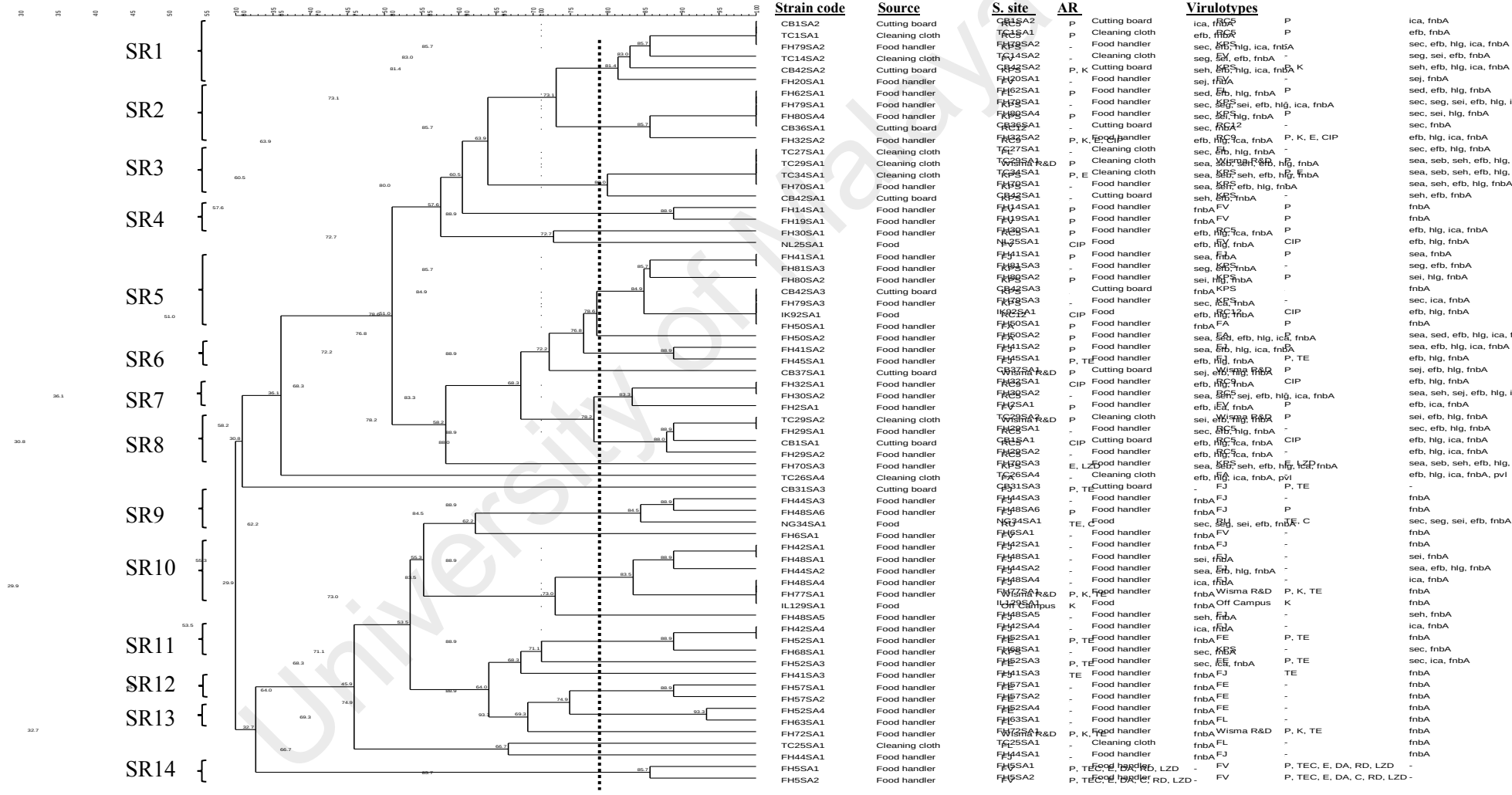


Figure 4.13: Representative REP-PCR gel photo of *Staph. aureus*
M1, 1 000 bp molecular ladder, M2, 100 bp molecular ladder; N, negative control; L1, TC1SA1; L2, FH30SA2; L3, CBISAI; L4, FH68SA1; L5, FH48SA6; L6, FH70SA2; L7, FH6SA1; L8, FH6SA2; L9, FH5SA1; L10, FH5SA2; L11, FH50SA1; L12, FH50SA2; L13, FH2SA1; L14, FH41SA3; L15, FH20SA1; L16, FH30SA1; L17, FH57SA2 were partial of the *Staph. aureus* isolated in this study.

Forty-seven REP-profiles (*D* value = 0.9762) were obtained of which 32 were unique profiles (Figure 4.14). Based on 80% similarity, 49 strains were subtyped into 14 clusters (size of 2 strains to 8 strains). The strains that were sampled from the same sampling site were closely related observed in SR9 and SR10. Cluster SR10 consisted of 3 closely related strains ($F < 0.89$) which were isolated from different food handlers' hands from



Strain code	Source	S. site	AR	Virulotypes
CB1SA2	Cutting board	RC5	P	ica, fnbA
TC1SA1	Cleaning cloth	RC5	P	efb, fnbA
FH79SA2	Food handler	KPS	-	sec, efb, hlg, ica, fnbA
TC14SA2	Cleaning cloth	FV	-	seg, sei, efb, fnbA
CB42SA2	Cutting board	KPS	P, K	seh, efb, hlg, ica, fnbA
FH20SA1	Food handler	FV	-	sej, fnbA
FH62SA1	Food handler	FJ	P	sed, efb, hlg, fnbA
FH79SA1	Food handler	KPS	-	sec, seg, sei, efb, hlg, ica, fnbA
FH80SA4	Food handler	KPS	P	sec, sei, hlg, fnbA
CB36SA1	Cutting board	KPS	-	sec, fnbA
FH32SA2	Food handler	RC9	P, K, E, CIP	efb, hlg, ica, fnbA
TC27SA1	Cleaning cloth	FC	-	sec, efb, hlg, fnbA
TC29SA1	Cleaning cloth	Wisma R&D	P	sea, seb, seh, efb, hlg, fnbA
TC34SA1	Cleaning cloth	KPS	P, E	sea, seb, seh, efb, hlg, fnbA
FH70SA1	Food handler	KPS	-	sea, seh, efb, hlg, fnbA
CB42SA1	Cutting board	KPS	-	seh, efb, fnbA
FH14SA1	Food handler	FV	P	fnbA
FH19SA1	Food handler	FV	P	fnbA
FH30SA1	Food handler	RC9	P	efb, hlg, ica, fnbA
NL25SA1	Food	FV	CIP	efb, hlg, fnbA
FH41SA1	Food handler	FJ	P	sea, fnbA
FH81SA3	Food handler	KPS	-	seg, sei, fnbA
FH80SA2	Food handler	KPS	P	sei, hlg, fnbA
CB42SA3	Cutting board	KPS		fnbA
FH79SA3	Food handler	KPS	-	sec, ica, fnbA
IK92SA1	Food	RC12	CIP	efb, hlg, fnbA
FH50SA1	Food handler	FA	P	fnbA
FH50SA2	Food handler	FA	P	sea, sed, efb, hlg, ica, fnbA
FH41SA2	Food handler	FJ	P	sea, efb, hlg, ica, fnbA
FH45SA1	Food handler	FJ	P, TE	efb, hlg, fnbA
CB37SA1	Cutting board	Wisma R&D	P	sej, efb, hlg, fnbA
FH32SA1	Food handler	RC9	CIP	efb, hlg, fnbA
FH30SA2	Food handler	RC9	-	sea, seh, sej, efb, hlg, ica, fnbA
FH2SA1	Food handler	FV	P	efb, ica, fnbA
TC29SA2	Cleaning cloth	Wisma R&D	P	sei, efb, hlg, fnbA
FH29SA1	Food handler	KPS	-	sec, efb, hlg, fnbA
CB1SA1	Cutting board	RC5	CIP	efb, hlg, ica, fnbA
FH29SA2	Food handler	RC5	-	efb, hlg, ica, fnbA
FH70SA3	Food handler	KPS	E, LZD	sea, seb, seh, efb, hlg, fnbA
TC26SA4	Cleaning cloth	FA	-	efb, hlg, ica, fnbA, pvl
CB31SA3	Cutting board	FJ	P, TE	-
FH44SA3	Food handler	FJ	-	fnbA
FH48SA6	Food handler	FJ	P	fnbA
NG34SA1	Food	RU	TE, C	sec, seg, sei, efb, fnbA
FH6SA1	Food handler	FV	-	fnbA
FH42SA1	Food handler	FJ	-	fnbA
FH48SA1	Food handler	FJ	-	sei, fnbA
FH44SA2	Food handler	FJ	-	sea, efb, hlg, fnbA
FH48SA4	Food handler	FJ	-	ica, fnbA
FH77SA1	Food handler	Wisma R&D	P, K, TE	fnbA
IL129SA1	Food	Off Campus	K	fnbA
FH48SA5	Food handler	FJ	-	seh, fnbA
FH42SA4	Food handler	FJ	-	ica, fnbA
FH52SA1	Food handler	FE	P, TE	fnbA
FH68SA1	Food handler	KPS	-	sec, fnbA
FH52SA3	Food handler	FE	P, TE	sec, fnbA
FH41SA3	Food handler	FJ	TE	fnbA
FH57SA1	Food handler	FE	-	fnbA
FH57SA2	Food handler	FE	-	fnbA
FH52SA4	Food handler	FE	-	fnbA
FH63SA1	Food handler	FL	-	fnbA
FH72SA1	Food handler	Wisma R&D	P, K, TE	fnbA
TC25SA1	Cleaning cloth	FL	-	fnbA
FH44SA1	Food handler	FJ	-	fnbA
FH5SA1	Food handler	FV	P, TEC, E, DA, RD, LZD	-
FH5SA2	Food handler	FV	P, TEC, E, DA, C, RD, LZD	-

AR, Antibiotic resistance

Figure 4.14: Dendrogram of *Staph. aureus* (REP-PCR*)* generated from UPGMA clustering method using dice coefficient analysis**.**

the same cafeteria. Besides that, there were strains isolated from different sources and different sampling sites were indistinguishable. In clusters SR3, SR8, SR12, SR13 and SR14, the strains exhibited similar virulence gene profiles within the cluster.

4.2.8 PFGE profiles of *Staph. aureus*

*Sma*I was used as the digestive enzyme to subtype *Staph. aureus*, 4 strains (5.0%) were untypeable. There were 9 bands to 14 bands generated from PFGE. The size of DNA fragments digested with *Sma*I was ranging from 30.3 kb to 700.0 kb (Figure 4.15).

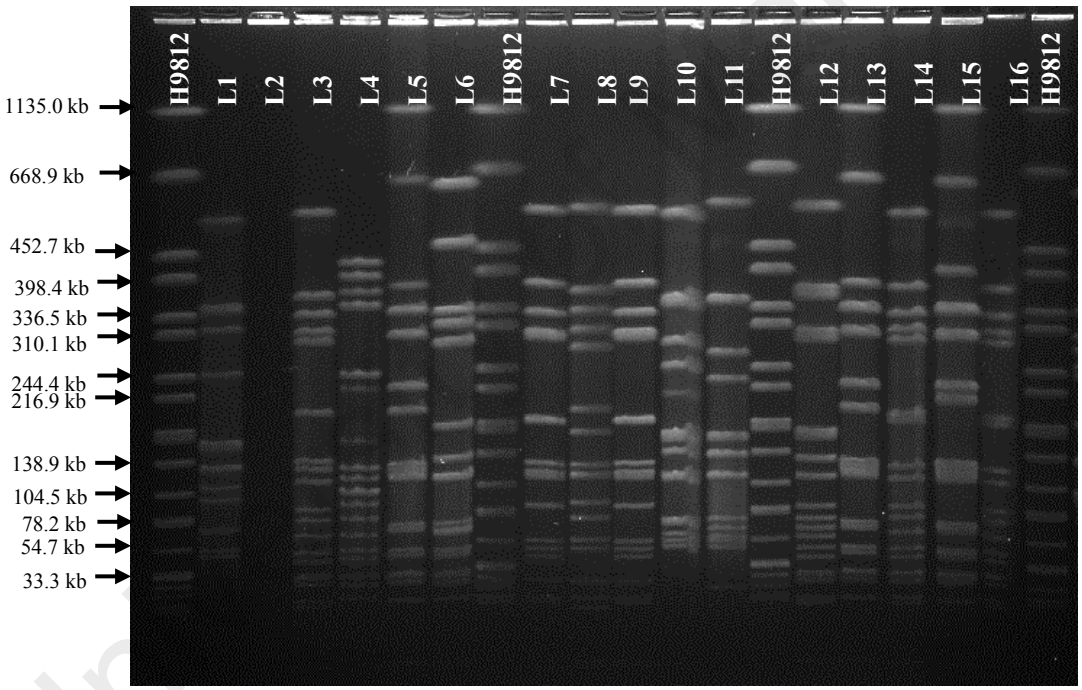
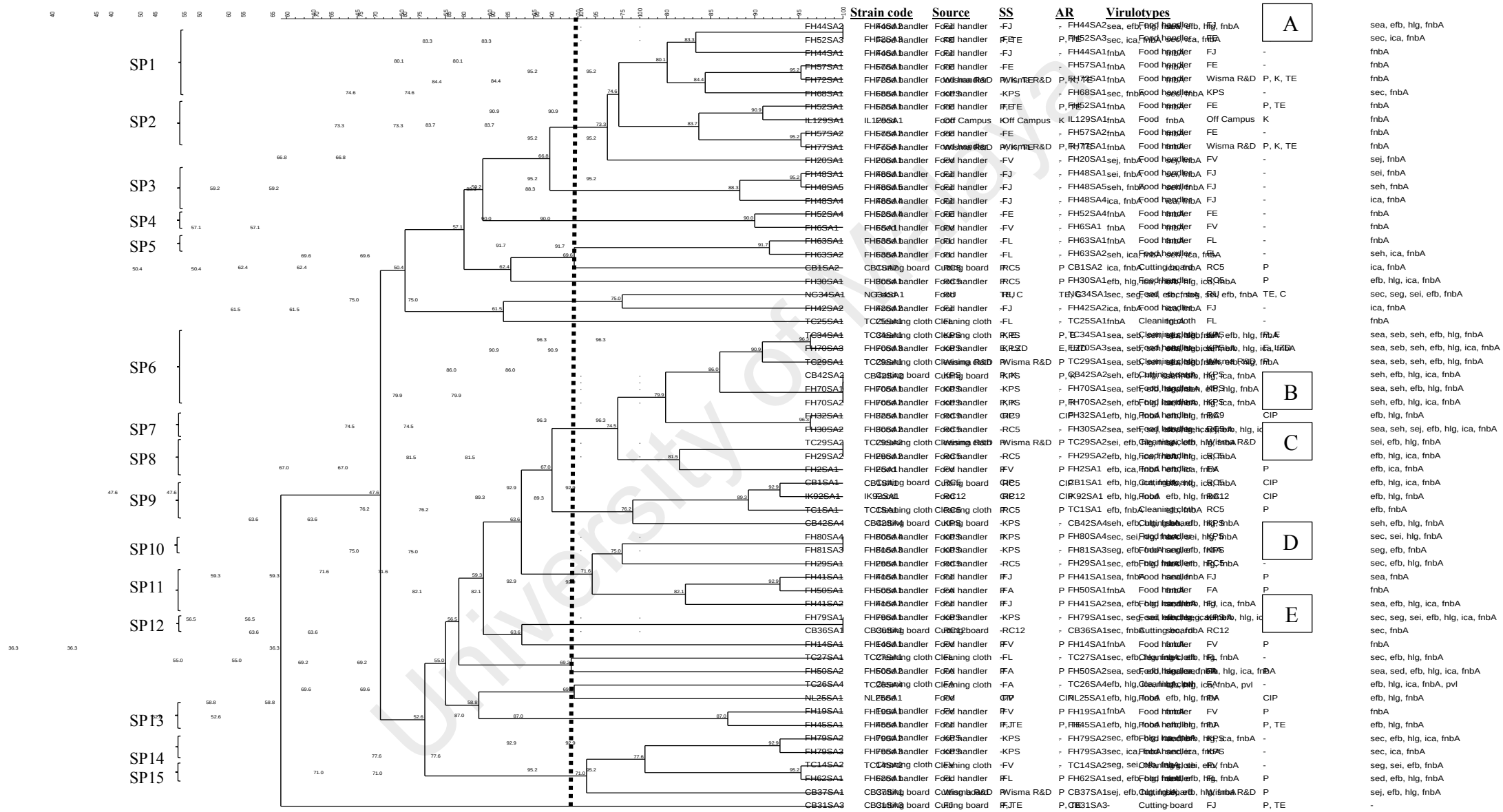


Figure 4.15: Representative PFGE gel photo of *Staph. aureus*
L1, FH52SA1; L2, FH57SA3; L3, CB42SA2; L4, FH30SA1; L5, FH79SA1; L6, NL25SA1; L7, FH29SA1; L8, FH29SA2; L9, FH29SA3; L10, FH68SA1; L11, FH48SA6; L12, FH2SA1; L13, FH79SA4; L14, TC34SA1 ; L15, CB37SA1; and L16, FH70SA3 were the *Staph. aureus* studied while H9812 was the universal reference standard, *S*. Braenderup.

Overall, PFGE produced 59 distinct reproducible banding patterns with *D* value = 0.9891 and *F* value = 0.36 to 1.00. Forty-two unique profiles were obtained. Duplicated strains were removed for cluster analysis (Figure 4.16). Analysis based on 80% similarity

showed that 59 strains were subtyped into 15 clusters (2 to 6 strains) and 19 distinctly different genetic profiles were observed. Five groups of groups of strains found instinguishable, denoted with alphabets “A”, “B”, “C”, “D”, and “E”. Similar virulotypes were observed within the cluster. The strains were relatively homogenous in the same sampling site which could be observed in clusters SP6 and SP9.



SS, Sampling site; AR, Antibiotic resistance

Figure 4.16: Dendrogram of *Staph. aureus* (PFGE*)* generated from UPGMA clustering method using dice coefficient analysis.

4.2.9 Antibiograms of *V. cholerae*

All *V. cholerae* strains were pan-susceptible to antibiotics tested except for ampicillin, erythromycin, nalidixic acids, and kanamycin. Approximately half were resistant to ampicillin (53.9%) while 100% of the strains were erythromycin-intermediate.

Table 4.11: Antibiograms of *V. cholerae*

Antibiotics (µg)	**No. of strain (n)**		
	Susceptible	**Intermediate**	**Resistant**
Tetracycline (30)	26	0	0
Ertapenem (30)	26	0	0
Amoxicillin-Clavulanic acid (10)	26	0	0
Ampicillin	12	0	14
Trimethoprim-sulfomethoxazole (25)	26	0	0
Ciprofloxacin (5)	26	0	0
Chloramphenicol (30)	26	0	0
Erythromycin (15)	0	26	0
Furazolidone (100mg)	26	0	0
Azithromycin (15)	25	1	0
Cefazolin (30)	26	0	0
Imipenem (10)	26	0	0
Aztreonam (30)	26	0	0
Nalidixic acid (30)	20	0	6
Kanamycin (30)	22	4	0
Ceftriaxone (30)	26	0	0

4.2.10 Virulence profiles of *V. cholerae*

The *V. cholerae* isolated was not harbouring *ctx*B which is the cholera toxin. Only 4 virulence genes were detected in the isolated *V. cholerae*: *rtx*C (n = 19), *hly*A (n = 14), *tox*R (n = 20) and *omp*W (n = 18). Figure 4.17 shows the virulence detection in *V. cholerae* by PCR method.

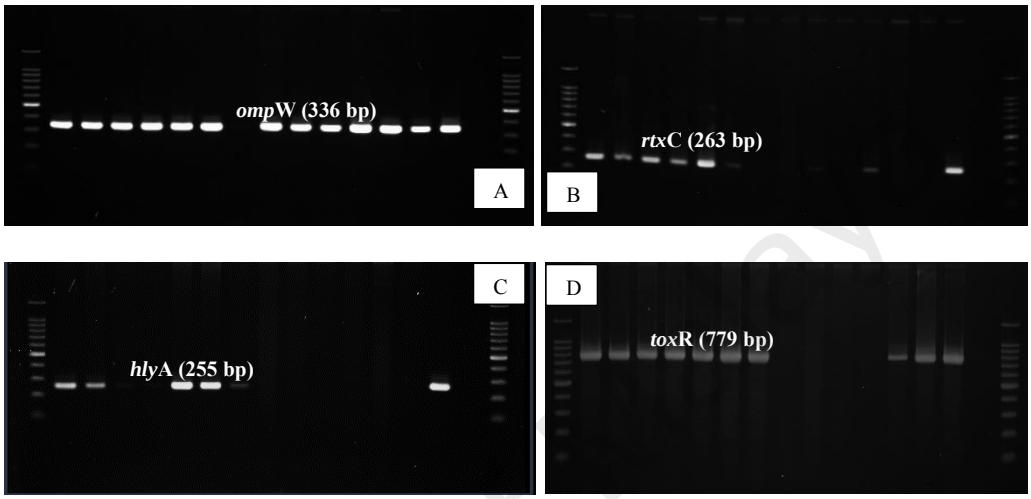


Figure 4.17: A series of representative gel pictures of virulotyping in *V. cholerae*. Gel photo (A) indicates the presence of *omp*W; (B) indicates the presence of *rtx*C; (C) indicates the presence of *hlg*A; and (D) indicates the presence of *tox*R in the *V. cholerae* isolates.

4.2.11 REP profiles of *V. cholerae*

There were 8 to 11 bands produced from REP-PCR. The amplified DNA were ranging from 200 bp to 3 000 bp. Twenty-six unrelated strains were subtyped into 7 distinct genetic profiles (F = 0.61 to 1.0). Besides three unique profiles, 23 strains were subtyped into 5 clusters based on 100% similarity. Duplicates were removed before determining the clonal relationship among the strains. Eight strains were subtyped into 3 clusters based on 75% similarity; D value = 0.8214. Strains isolated from the same source were subtyped into the same cluster, Cluster VR1 and VR2 (Figure 4.18). Similar virulotypes and resistotypes were observed within the cluster.

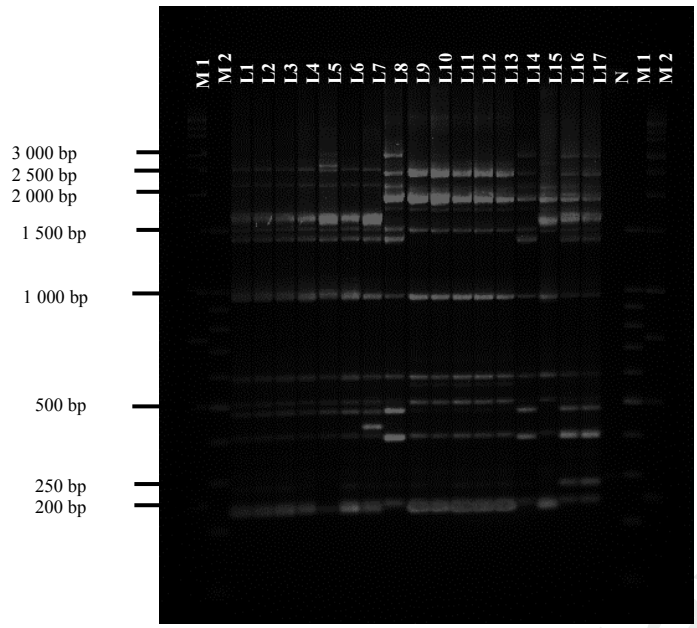
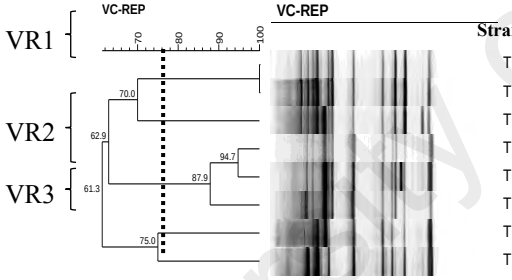


Figure 4.18: Representative REP-PCR picture for *V. cholerae* subtyping.
M1, 1 000 bp molecular ladder, M2, 100 bp molecular ladder; L1, TC3CV1; L2, TC3CV5; L3, TC3CV6; L4, TC3CV7; L5, TC3CV8; L6, TC3CV9;L7, TC10CV1; L8, TC13CV1; L9, TC13CV3; L10, TC13CV4; L11, TC13CV6; L12, TC13CV7; L13, TC13CV13; L14, TC16CV1; L15, TC17CV2; L16, TC18CV1; and L17, TC18CV6 were the *V. cholerae* isolated in this study.



Strain code	Source	S. site	Resistotypes	Virulotypes
TC16CV1	Cleaning cloth	FV	-	toxR, rtxC, hlyA, ompW
TC13CV1	Cleaning cloth	FV	-	toxR, rtxC, ompW
TC18CV1	Cleaning cloth	FJ	-	hlyA, ompW
TC3CV1	Cleaning cloth	RC12	AMP, NA	toxR, rtxC, hlyA, ompW
TC10CV1	Cleaning cloth	RC11	AMP	toxR, rtxC, hlyA, ompW
TC3CV8	Cleaning cloth	RC12	AMP	toxR, rtxC, ompW
TC17CV2	Cleaning cloth	FJ	-	toxR, rtxC, hlyA
TC13CV3	Cleaning cloth	FV	-	toxR, hlyA, ompW

Figure 4.19: Dendrogram of *V. cholerae* (REP-PCR*)* generated from UPGMA clustering method using dice coefficient analysis.

4.2.12 PFGE profiles of *V. cholerae*

V. cholerae strains were 100% typeable by PFGE. It produced banding patterns ranging from 16 to 21 bands (F = 0.58-1.00) with the DNA size of 28.8 kb to 336.5 kb (Figure 4.20). PFGE subtyped 26 strains into 13 genetic profiles. Clustering based on 75% similarity, 13 non-duplicate strains were subtyped into 4 clusters with D value = 0.859. Two strains, TC10CV1 and TC3CV8 were not clustered. The strains that were isolated from the same sampling site were subtyped into the same cluster (VP1, VP2,

VP3, and VP4). The virulotypes and resistotypes were almost similar among the strains within the cluster (Table 4.21).

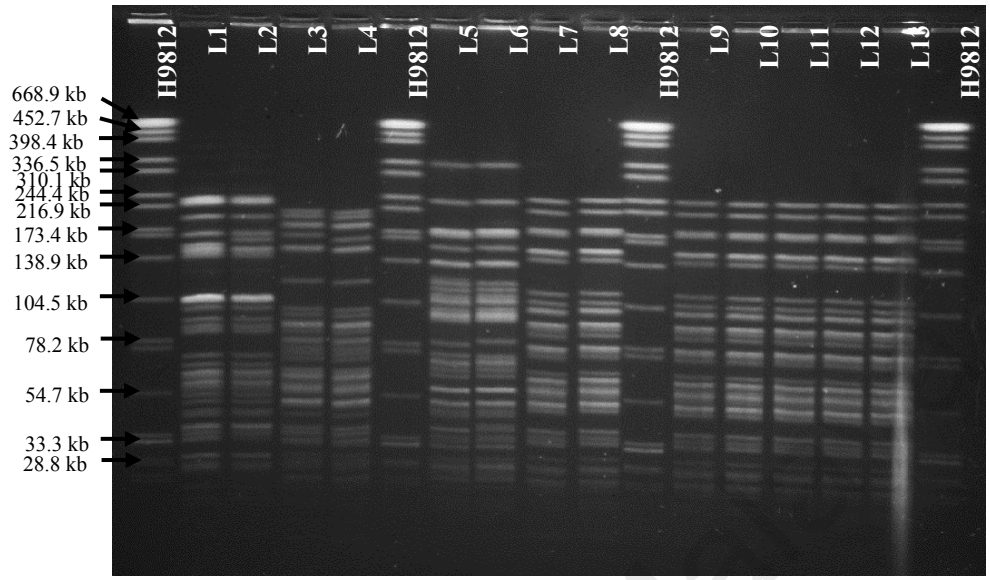


Figure 4.20: Representative PFGE gel picture for *V. cholerae* subtyping. L1, TC13CV7; L2, TC13CV13; L3, TC16CV1; L4, TC16CV2; L5, TC17CV2; L6, TC17CV20; L7, TC18CV1; L8, TC18CV6; L9, TC18CV7; L10, TC18CV8; L11, TC18CV9; L12, TC18CV10; and L13, TC18CV2 were *V. cholerae* strains studied while H9812 was the universal reference standard, *S*. Braenderup.

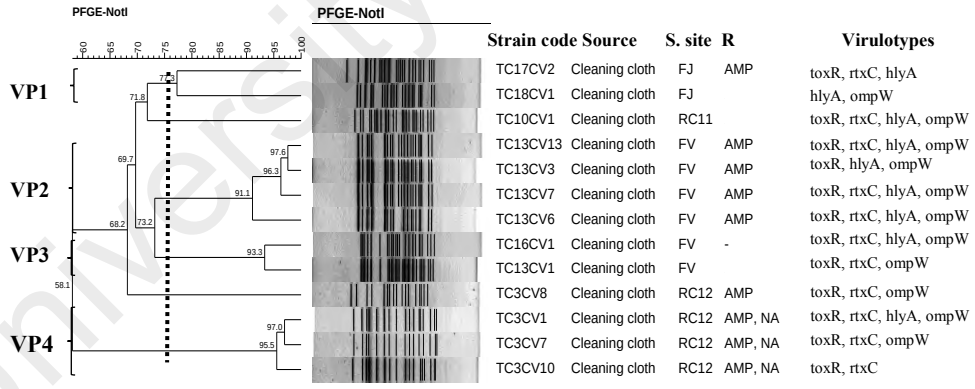


Figure 4.21: Dendrogram of *V. cholerae* (PFGE*)* generated from UPGMA clustering method using dice coefficient analysis.

4.3 Food safety knowledge, attitudes, and practices of the food handlers

4.3.1 Socio-demographic characteristics of the food handlers

The questionnaire was conducted on the food handlers on a voluntary basis. A total of 67 questionnaires had been collected from food handlers who worked in the cafeterias at the University. The respondents consisted of 40% male and 60% female food handlers (Table 4.12). The majority of the food handlers were aged between 21 to 41 year old (n = 43, 64.2%). They were mainly non-Malaysians (61%, n = 41), the majority were Indonesians. Only 16% (n = 11) of the respondents had no formal education. The managers or supervisors and cashiers participated in this questionnaire were categorised as 'others' in the 'role' section. About 46% of the food handlers were newly enrolled in food service (≤ 2 years' experience). Although the food safety training is compulsory for all the food handlers but there were still 25% food handlers who did not attend any food safety training.

4.3.2 Knowledge, attitude, and practices of food handlers

The mean of total knowledge score was 61.7 ± 8.1 %. Of 6 constructs in the knowledge section, the respondents had a high score for personal hygiene. They were aware of the importance of hand washing during food handling (100%) or after touching body parts (87%) and using toilet (99%). However, the food handlers had poor knowledge on foodborne pathogens and knowledge on cross-contamination sanitation and prevention. Only 10.8% of the respondents were aware that washing cutting boards with just water and soap were not enough to clean the cutting boards. However, they knew that the cutting board and knife that were used to cut raw vegetables and meats should be separated (81%). Besides that, 48% (n = 32) of the respondents did not know that they could thaw the frozen meat in the refrigerator, but 82% (n = 55) of them knew that they could thaw the frozen meat under running tap. As mentioned, most of the respondents lacked the

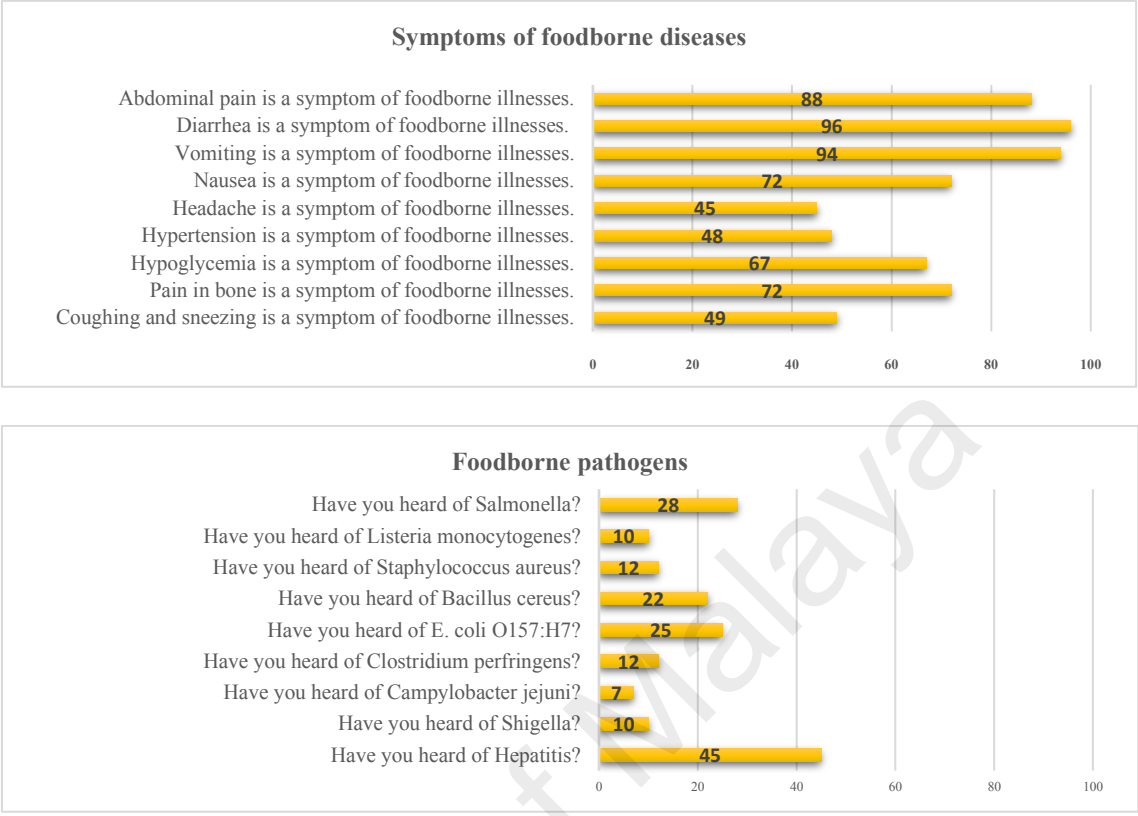
knowledge on foodborne pathogens. Hepatitis virus was the most well-known pathogens among these respondents (43.2%), followed by *Salmonella* spp. (25.4%) and *E. coli* O157:H7 (22.4%). Almost half (46%, n = 31) reported that they did not know any of the foodborne pathogens listed (Figure 4.22).

Overall, the respondents showed positive attitudes and good practices during food handling. The mean attitude score was 51.9 (total = 57) while the average score for self-reported practices was 53.2 out of 60. Table 4.13 and Table 4.14 described the responses of the respondents on attitudes and self-reported practices in regards to food safety, respectively. The respondents were willing to read more, attend more food safety training courses, seminars to improve their knowledge on food safety. Furthermore, they also agreed that maintaining clean cooking environment, alert of food safety issues and self-checking are the paramount ways in ensuring food safety. Most of the respondents claimed that they use gloves and caps during food handling and frequently washed their hands during food preparation. However, about 27% (n = 18) food handlers revealed that they had never worn a mask when they were handling unwrapped foods. Apart from that, only 63% (n = 42) of the respondents did not work when they are sick.

Table 4.12 Participants' demographic characteristics.

Variable	Item	Number	Percent (%)
Gender	Male	27	40.3
	Female	40	59.7
Age	< 21 years old	5	7.5
	21-41 years old	43	64.2
	> 41 years old	19	28.4
Nationality	Malaysian	26	38.8
	Foreigner	41	61.2
Marital Status	Single	14	20.9
	Married	49	73.1
	Divorce	4	6.0
Education level	No formal education	11	16.4
	Primary school	7	10.5
	Secondary school	37	55.2
	College/ University	12	17.9
Work experience	< 2 years	31	46.3
	2-4 years	16	23.9
	4-6 years	9	13.4
	> 6 years	11	16.4
Job responsibility	Cooking	35	52.3
	Cleaning and washing dishes	3	4.5
	Serving food	17	25.4
	Preparation of food ingredients	4	6.0
	Others (cashier, manager, etc.)	8	11.9
Did you attend the Safe Food Handling course?	No	17	25.4
	Yes	50	74.6
When did you attend the Safe Food Handling course?	Never attend before	17	25.4
	≤ 3 years ago	29	43.3
	> 3 years ago	21	31.3
Total		**67**	**100**

Figure 4.22: Percentage of correct answers on food safety knowledge scored by 67 food handlers.



(except for construct of foodborne pathogens, in which the bars indicate percentage of "yes" response.)*

Figure 4.22, continued.

Table 4.13: Participant food safety attitude scores.

Construct	Item	Mean	SD	Min	Max
Self-improvement					
	I would read more journals about food safety in order to enhance my food sanitation knowledge.	3.48	0.88	1.00	4.00
	I think by attending a sanitation seminar, it would increase my sanitation knowledge and ideas.	3.90	0.43	1.00	4.00
	I would attend a cooking or service competition to improve my professional knowledge.	3.16	1.18	1.00	4.00
	I would attend food safety seminar to gain more food safety knowledge.	3.82	0.52	1.00	4.00
	I think I do not need to attend food safety seminar because I think I have sufficient knowledge about food safety.	1.57	1.58	1.00	4.00
Food safety concern					
	Food handlers are responsible to prevent food poisoning.	3.90	0.53	1.00	4.00
	Government is responsible to prevent food poisoning.	3.15	1.28	1.00	4.00
	University is responsible to prevent food poisoning.	3.27	1.18	1.00	4.00
	Consumers are responsible to prevent food poisoning.	3.39	1.10	1.00	4.00
	Maintaining a clean cooking environment is a good way to control food safety.	3.96	0.27	2.00	4.00
	Self-checking of food safety is important to restaurants and institutions.	3.81	0.43	2.00	4.00
	Food safety is more important than taste.	3.81	0.43	2.00	4.00
	Food safety knowledge is important to ensure food is prepared in a safe manner.	3.85	0.47	1.00	4.00
	Food poisoning is not a serious matter.	1.69	1.26	1.00	4.00

Table 4.14: Participant self-reported food safety practices.

Item	Mean	SD	Min	Max
Do you wash your hands before touching unwrapped raw foods?	4.58	0.78	1.00	5.00
Do you wash your hands after touching unwrapped raw foods?	4.64	0.85	1.00	5.00
Do you use gloves when you touch or distribute unwrapped foods?	4.33	1.15	1.00	5.00
Do you use protective clothing (apron) when you touch or distribute unwrapped foods?	4.55	0.99	1.00	5.00
Do you use mask when you touch or distribute unwrapped foods?	2.76	1.47	1.00	5.00
Do you use cap when you touch or distribute unwrapped foods?	4.48	1.20	1.00	5.00
Do you use different chopping board for raw meat and fresh produce (vegetables and fruit)?	4.33	1.12	1.00	5.00
Do you wash and sanitize the working clothes?	4.81	0.58	1.00	5.00
Do you use a different cloth or towel to dry plates?	4.61	0.95	1.00	5.00
Do you wash and sanitize the knife after chopping raw chicken or meat?	4.84	0.37	4.00	5.00
Do you use clean and washed plate for RTE foods?	4.93	0.26	4.00	5.00
Do you work when you are sick (flu, cold, diarrhoea, coughing, etc.)?	4.31	1.05	1.00	5.00

4.3.3 Attribution of KAP scores to different categories

This study shows that the education level, working experience and safe food handling course significantly influenced the food safety knowledge and attitudes of food handlers ($p < 0.05$; Table 4.15). A higher education level did not necessarily lead to a better knowledge and attitudes on food safety. The food handlers who had more working experience in food service had better overall knowledge (more than 6 years> 4-6 years > 2-4 years ≥ 2 years, $p < 0.05$). Also, food handlers who attended safe food handling course had significantly improved knowledge (especially on foodborne pathogens) and attitudes ($p < 0.05$).

4.3.4 Correlation among food handlers' knowledge, attitude and practices of food safety

The correlation of knowledge and attitudes ($r_s = -0.009$, $p = 0.942$), knowledge and practices ($r_s = 0.170$, $p = 0.169$) and attitudes and practices ($r_s = -0.122$, $p = 0.327$) were insignificant. In other words, knowledge of the food handlers may not affect the attitudes and practices in food handling.

Table 4.15: Attribution of food safety knowledge, attitude and self-reported scores to educational level, work experience, and safe food handling course of participants (n = 67).

	Education level				Work Experience				Did you attend the Safe Food Handling course?		When did you attend the Safe Food Handling course?		
	No formal education	**Primary school**	**Secondary school**	**College/ University**	**≤ 2 years**	**2-4 years**	**4-6 years**	**> 6 years**	**No**	**Yes**	**Never attended**	**≤ 3 years ago**	**> 3 years ago**
	(n = 11)	**(n = 7)**	**(n = 31)**	**(n = 12)**	**(n = 31)**	**(n = 12)**	**(n = 9)**	**(n = 11)**	**(n = 17)**	**(n = 50)**	**(n = 17)**	**(n = 29)**	**(n = 21)**
Knowledge (%)													
Personal hygiene	95.45$_{a}$	87.14$_{a}$	94.32$_{a}$	88.33$_{a}$	92.26$_{a}$	91.25$_{a}$	94.44$_{a}$	94.55$_{a}$	90.00$_{a}$	93.60$_{a}$	90.00$_{a}$	94.83$_{a}$	91.90$_{a}$
Cross contamination prevention and sanitation	49.24$_{a}$	60.71$_{a}$	49.77$_{a}$	53.47$_{a}$	50.54$_{a}$	53.13$_{a}$	50.93$_{a}$	52.27$_{a}$	48.04$_{a}$	52.67$_{a}$	48.04$_{a}$	53.45$_{a}$	51.59$_{a}$
Food handling	**66.36$_{a,b}$**	**52.86$_{a}$**	**75.14$_{b}$**	**74.17$_{b,c}$**	**73.23$_{a,b}$**	**61.25$_{a}$**	**66.67$_{a,b}$**	**83.64$_{b}$**	73.53$_{a}$	70.40$_{a}$	73.53$_{a}$	68.28$_{a}$	73.33$_{a}$
Health problems that would affect food safety	62.73$_{a}$	54.29$_{a}$	69.19$_{a}$	68.33$_{a}$	61.94$_{a}$	63.13$_{a}$	73.33$_{a}$	78.18$_{a}$	72.94$_{a}$	64.20$_{a}$	72.94$_{a}$	60.00$_{a}$	70.00$_{a}$
Symptoms of food borne diseases	78.79$_{a}$	66.67$_{a}$	66.07$_{a}$	75.93$_{a}$	66.67$_{a}$	72.22$_{a}$	70.37$_{a}$	75.76$_{a}$	66.01$_{a}$	71.33$_{a}$	66.01$_{a}$	68.58$_{a}$	75.13$_{a}$
Food borne pathogens	**6.06$_{a}$**	**25.40$_{a,b}$**	**16.22$_{a}$**	**38.89$_{b}$**	14.70$_{a}$	19.44$_{a}$	19.75$_{a}$	33.33$_{a}$	4.58$_{a}$	24.67$_{b}$	**4.58$_{a}$**	**23.75$_{b}$**	**25.93$_{b}$**
Overall knowledge score	60.0$_{a}$	58.3$_{a}$	62.1$_{a}$	66.4$_{a}$	**60.2$_{a}$**	**60.3$_{a}$**	**62.8$_{a,b}$**	**69.5$_{b}$**	59.6$_{a}$	63.0$_{a}$	59.6$_{a}$	61.7$_{a}$	64.7$_{a}$
Food safety attitude													
Self-improvement	**3.52$_{a,b}$**	**3.46$_{a,b}$**	**3.74$_{a}$**	**3.25$_{b}$**	3.56$_{a}$	3.52$_{a}$	3.47$_{a}$	3.89$_{a}$	3.62$_{a}$	3.58$_{a}$	3.62$_{a}$	3.61$_{a}$	3.54$_{a}$
Food safety concern	3.46$_{a}$	3.63$_{a}$	3.36$_{a}$	3.46$_{a}$	3.35$_{a}$	3.50$_{a}$	3.53$_{a}$	3.42$_{a}$	3.14$_{a}$	3.52$_{b}$	**3.14$_{a}$**	**3.53$_{b}$**	**3.50$_{b}$**
Overall attitude score	3.48$_{a}$	3.58$_{a}$	3.48$_{a}$	3.40$_{a}$	3.41$_{a}$	3.50$_{a}$	3.51$_{a}$	3.57$_{a}$	3.29$_{a}$	3.54$_{b}$	**3.29$_{a}$**	**3.56$_{b}$**	**3.51$_{a,b}$**
Self-reported practices													
Overall practices score	4.33$_{a}$	4.15$_{a}$	4.23$_{a}$	4.06$_{a}$	4.27$_{a}$	4.16$_{a}$	4.02$_{a}$	4.28$_{a}$	4.29$_{a}$	4.18$_{a}$	4.29$_{a}$	4.21$_{a}$	4.14$_{a}$

Values in the same row and subtable not sharing the same subscript are significantly different at $p < .05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances and are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.

4.4 Self-reported practices versus microbial contamination on food handlers' hands

The food handlers who had a non-compliance count of coliforms (> 1.3 log CFU/cm^2) claimed to use gloves more frequently when touching or distributing unwrapped foods than those who had a compliance count of coliform (Table 4.16). On the contrary, the food handlers who were detected with the presence of *Salmonella* attested that they were less frequent in using caps while handling food (4.2 ± 1.6, n = 24, $p < 0.05$) than those who had negative detection for in the presence of *Salmonella*. Furthermore, the respondents who had of an exceeded limit of aerobic colony count (≥ 20 CFU/cm^2) on their hands declared that they wash their hands before touching the unwrapped foods (4.7 ± 0.5, n = 27, $p < 0.05$) more frequently. The food handlers who has coliform count exceeded the threshold reported that they sanitise their working cloths more frequently (5.0 ± 0.0, n = 15, $p < 0.05$).

Table 4.16: Self-reported practices vs. microbial contamination on food handlers' hands.

Item	**Aerobic colony count**[a]		**Coliform**[a]		***E. coli***[a,b]	***Staph. aureus***[a]		***Salmonella***[a]	
	≥ Threshold (n = 27)	< Threshold (n = 14)	≥ Threshold (n = 15)	< Threshold (n = 26)	< Threshold (n =41)	≥ Threshold (n = 2)	< Threshold (n = 39)	Detected (n = 24)	Not detected (n = 17)
Do you wash your hands before touching unwrapped raw foods?	**4.7 ± 0.5**	**4.1 ± 0.9**	4.7 ± 0.5	4.4 ± 0.9	4.5 ± 0.7	5.0 ± 0.0	4.5 ± 0.8	4.7 ± 0.6	4.4 ± 0.8
Do you wash your hands after touching unwrapped raw foods?	4.6 ± 1.0	4.6 ± 0.9	4.4 ± 1.2	4.6 ± 0.8	4.6 ± 1.0	5.0 ± 0.0	4.5 ± 1.0	4.4 ± 1.2	4.6 ± 0.8
Do you use gloves when you touch or distribute unwrapped foods?	4.3 ± 1.2	3.9 ± 1.2	**4.7 ± 0.7**	**3.9 ± 1.4**	4.2 ± 1.2	5.0 ± 0.0	4.2 ± 1.2	4.2 ± 1.4	4.2 ± 1.1
Do you use protective clothing (apron) when you touch or distribute unwrapped foods?	4.4 ± 1.2	4.6 ± 0.7	4.1 ± 1.5	4.7 ± 0.7	4.5 ± 1.1	5.0 ± 0.0	4.5 ± 1.1	4.3 ± 1.2	4.6 ± 1.0
Do you use mask when you touch or distribute unwrapped foods?	2.6 ± 1.4	2.5 ± 1.2	3.1 ± 1.5	2.3 ± 1.1	2.6 ± 1.3	3.5 ± 0.7	2.5 ± 1.3	2.6 ± 1.4	2.8 ± 1.3
Do you use cap when you touch or distribute unwrapped foods?	4.7 ± 1.1	4.6 ± 1.1	4.7 ± 1.0	4.7 ± 1.1	4.7 ± 1.1	5.0 ± 0.0	4.6 ± 1.1	**4.2 ± 1.6**	**4.9 ± 0.3**
Do you use different chopping board for raw meat and fresh produce (vegetables and fruit)?	4.3 ± 1.2	4.0 ± 0.9	4.4 ± 1.2	4.1 ± 1.1	4.2 ± 1.1	5.0 ± 0.0	4.2 ± 1.1	4.5 ± 0.8	4.2 ± 1.1
Do you wash and sanitize the working clothes?	4.9 ± 0.3	4.4 ± 1.1	**5.0 ± 0.0**	**4.6 ± 0.9**	4.7 ± 0.7	5.0 ± 0.0	4.7 ± 0.7	4.8 ± 0.4	4.6 ± 0.9
Do you use a different cloth or towel to dry plates?	4.5 ± 1.2	4.3 ± 1.1	4.7 ± 1.0	4.3 ± 1.2	4.4 ± 1.2	5.0 ± 0.0	4.4 ± 1.2	4.6 ± 0.9	4.5 ± 1.1
Do you wash and sanitize the knife after chopping raw chicken or meat?	4.9 ± 0.3	4.4 ± 0.5	**4.9 ± 0.3**	**4.7 ± 0.5**	4.8 ± 0.4	5.0 ± 0.0	4.8 ± 0.4	4.8 ± 0.4	4.7 ± 0.5
Do you use clean and washed plate for RTE foods?	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.3	5.0 ± 0.0	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.3
Do you work when you are sick (flu, cold, diarrhoea, coughing, etc.)?	4.3 ± 1.2	3.6 ± 1.1	**4.5 ± 0.7**	**3.8 ± 1.3**	4.0 ± 1.2	3.0 ± 2.8	4.1 ± 1.1	4.2 ± 1.3	4.0 ± 1.1

Results are based on two-sided tests assuming equal variances with significance level .05. The significant pair is highlighted in bold. Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction. The value after "±" indicates the standard deviation of the respective response.

[a] Threshold of aerobic bacteria count, coliforms, *E. coli* and *Staph. aureus* were based on Tan *et al*., 2013 and Sneed *et al*., 2004, which is ≥ 1.3 log CFU/cm^2.

[b] T-test was not conducted as the one of the sample size is too little for confident statistical analysis.

82

CHAPTER 5: DISCUSSION

Food safety has always been a hot topic in Malaysia. Wide varieties of foods are sold in Malaysia due to our diverse cultures. Thus, patronising restaurants and cafeterias in Malaysia is a common culture. Food safety encompasses many aspects. One of the aspects is the knowledge of the types of microbial contamination which are well studied. However, we need to acquire more knowledge on the microbial contamination of the factors associated with RTE foods, understand the characteristics and the possible dissemination routes of the foodborne pathogens, and the knowledge, attitudes, and practices of food handlers towards food safety.

5.1 Poor microbiological quality of RTE foods, FCS, TCC and food handlers' hands

It is commonly assumed that foods with raw ingredients such as raw vegetables and fruits carry a higher risk of microbial contamination; and the results of this study concurred with this notion, in which 61.5 % (n = 8) of the samples in food group D (Table 4.2) were positive for *Salmonella* DNA. Also, food groups C and D that contain raw ingredients recorded the highest non-compliance rate based on aerobic colony count (ACC) and total coliforms (food group C, 62.5%; food group D, 69.2%). The prevalence of *Salmonella* DNA in RTE foods reported in this study might seem to be a little higher than the report from Pui *et al.* (2011) who reported 23.3% of the sliced fruits were detected positive for *Salmonella*. It was also noted that the MPN-PCR method detected a higher percentage of *Salmonella* compared to the culturing method, for instance: *Salmonella* was present and isolated from 0.2% of the RTE foods sampled from the United Kingdom, (Sagoo et al., 2003); 1.2% from Brazil (Oliveira et al., 2011); and 8.0%, Turkey (Gurler, et al., 2015). The presence of *Salmonella* in local foods is not surprising (Arumugaswamy et al., 1995; Modarressi & Thong, 2010). Although *Salmonella* has been widely linked to

poultry, Noorzaleha et al. (2003) had highlighted the potential risk of *Salmonella* transmission through consumption of raw vegetables. It is interesting to note that, in this study, the occurrence of *Salmonella* was significantly higher in cold drinks with milk (food group G) ($p = 0.045$, df = 7, $\chi^2 = 14.372$). It is believed that the possible sources of contamination might be contaminated ice cubes and water to prepare the beverages (Noor Izani et al., 2012), as well as cross-contamination from food handlers. During sample collection, the condensed milk was observed left uncovered on the counter top which made it prone to contamination. Not many studies had focused on how the milk added to the beverages provides a better condition for the persistence and even growth of *Salmonella* spp. but dairy products are frequently associated with contamination of pathogenic bacteria. Hence, more surveillance and studies should be conducted to investigate if cold beverages with milk could pose a higher risk to the consumer. Also, the source of contamination, specifically ice cubes must be revealed.

In this study, foods that contain raw ingredients (food group C and D) were found to demonstrate significantly ($p < 0.05$) lower microbiological quality based on ACC and coliform count (Table 4.2). In food group C, 93.8% of the food was at the unsatisfactory level based on ACC. This finding concurred with the reports of Oliveira *et al.* (2011) in which 96% of the minimally processed vegetable samples contained > 5 log CFU/g of ACC. The uncooked foods were probably contaminated prior to cooking and the insufficient cooking time could have further encouraged the growth of more microorganisms in this type of food. It is noteworthy to point out that the occurrences of ACC and coliform (> 5 log CFU/ml) in cold drinks (food group G and H) were higher than hot beverages (food group E and F) and again, ice cubes could be the source of contamination (Noor Izani et al., 2012). Surprisingly 11.8% and 8.3% of samples in food group E (hot beverage with milk) and F (hot beverages without milk) harboured > 4 log

CFU/mL of ACC. This might be due to the cross contamination of the utensils that were used to prepare drinks or due to the food handler's hands.

In this study, FCS and TCC in the food premises were sampled for microbiological analysis to reveal their roles as potential vehicles for bacteria transmission. Studies investigating the transmission of pathogens through FCS and TCC are limited. Cunningham *et al.* (2011) reported that 70.3% of the visually clean FCS samples failed in adenosine triphosphate bioluminescence assessment. Bacteria on the FCS are highly possible to be transferred onto the raw or RTE foods. Willis *et al.* (2013) also demonstrated that one-third of the FCS samples that they obtained were of an unsatisfactory level of hygiene while more than half of the cleaning cloth samples (56%; n = 98) failed in the microbiological quality assessment. During the observation, some food handlers left their cleaning cloths on the clean plates, and this could constitute a potential route of transmission of microbial contaminants and poses a potential health risk to consumers.

Food handler plays a key role in food safety as they are highly involved in food preparation and food serving. Microbial assessments enable a better insight of the current food safety practices in food premises. The microbiological hygiene assessment will reflect the real practices of proper safe food handling. The findings from this study were not promising as many food handlers were found to have microbial counts exceeding the standards (Table 3.2). More importantly, *Salmonella* DNA was detected from the hands of participated food handlers (n = 41, 48%). The *Salmonella* present could be due to cross-contamination with TCC or FCS. Besides that, these food handlers could be the asymptomatic carriers for *Salmonella* transmission as *Salmonella* can remain as carrier state up to 300 days after infection (Todd et al., 2007). It raises a lot of public health concern as most of the food handlers were not wearing gloves during food handling, as

observed during the study. This could eventually increase the risk of foodborne diseases, especially when they are dealing with RTE foods.

The recovery rate of the *Salmonella* spp., *V. cholerae,* and *V. parahaemolyticus* was low. This may be due to the sublethally injured cells could not be revived after enrichment. *Salmonella* spp. (Sinton, 2006) and *Vibrio* spp. (Colwell & Grimes, 2000) are common bacteria that have viable but non-culturable (VBNC) stage. Even though these bacteria were in VBNC stage but they are still capable of causing infection and revert to be culturable once it is resuscitated in a favourable condition (Smith et al., 2008). On the other hand, Busse (1995) emphasised that the recovery of *Salmonella* can still apt to failure because it can be degraded during enrichment step although the contaminated parts have been drawn. Pui *et al.* (2011) were able to recover only 9.5% *Salmonella* from the MPN-PCR positive food samples. However, we could isolate *V. cholerae* from the TCC with the recovery rate of 27%.

5.2 High number of multidrug resistant *E. coli* and virulent *Staph. aureus* isolated from RTE foods, FCS, TCC and food handlers' hands

The emergence of multidrug-resistant bacteria has been the major problem in treating bacterial infections. CDC (2013a) estimated 2 049 442 cases of illnesses were caused by antimicrobial resistance and 23 000 were associated with deaths. The surveillance data from European Food Safety Authority (2010) shows that the antimicrobial resistance in *E. coli* towards the drugs used in human and veterinary medicine has been perpetually the highest.

In this study, the drug-resistance in *E. coli* was high, particularly the resistance to tetracycline and erythromycin. The Institute for Medical Research, IMR (2014) had reported similar findings in which the resistance to tetracycline was the highest at 47.4%. The same finding was reported by Tadesse *et al.* (2012) of which the *E. coli* isolated from humans and food animals were highly resistant tetracycline. Tetracycline was introduced

in the 1940s and the first tetracycline-resistant bacteria was isolated in 1953 (Chopra & Roberts, 2001). Since then, the prevalence of tetracycline-resistance had been increased, regardless in pathogenic bacteria, opportunistic pathogens or commensals (Chopra & Roberts, 2001). Several studies in Malaysia on the antimicrobial resistance of *E. coli* also showed resistance tetracycline was remained high in these studies (Alhaj et al., 2007; Lim et al., 2009).

Besides resistance towards tetracycline and erythromycin, penicillin-resistant, quinolones-resistant, aminoglycosides-resistant and phenicol-resistant were observed. Quinolones-resistant *E. coli* was discovered after the introduction in the late 1980s (Hooper & Wolfson, 1993; Webber & Piddock, 2001). Unlike other studies, quinolones-resistant *E. coli* was frequently isolated from food animals (Johnson et al., 2006; Sáenz et al., 2001; Thorsteinsdottir et al., 2010) and humans (Garau et al., 1999; Melo et al., 2015), but majority of the quinolones-resistant *E. coli* in this study were isolated from RTE foods. This finding suggested that quinolone-resistant *E. coli* from food could be originated from poultry products.

Since the antimicrobial resistance towards erythromycin was high, so it is not surprising to see high co-resistance of tetracycline with erythromycin (98.7%, 76 out of 77). The co-resistance between tetracycline with both ampicillin and trimethoprim-sulfamethoxazole (45.5%, n = 35) were secondly high after erythromycin. This scenario was also reported in the study by Tadesse et al. (2012).

Although none of the *E. coli* isolates was resistant to carbapenems, but an *E. coli* isolated from cutting board was an extended spectrum beta-lactamase (ESBL)-producing *E. coli*. The information on ESBL-producing *E. coli* in RTE food is scant. ESBL-producing *E. coli* was never isolated from RTE foods, but it was often associated with poultry products (Geser et al., 2012; Johnson et al., 2007; Slama et al., 2010). The antimicrobial resistance could have been emerged from food animals, especially poultry

(Johnson et al., 2007). The isolation of ESBL-producing *E. coli* from cutting board could be disseminated from poultry products or vegetables onto the cutting board. However, it lacks information on the foods prepared on the cutting board in which the ESBL-producing *E. coli* was isolated. Since the *E. coli* isolates did not carry any virulence genes, thus it could be just a commensal.

No methicillin-resistant *Staph. aureus* (MRSA) was found in this study. The high number of penicillinase–producing *Staph aureus* were obtained, mainly from food handlers. This finding is not surprising as in penicillin-resistant *Staph. aureus* are now widely spread in the community (Appelbaum, 2007; Chambers, 2001) after the introduction of penicillin in treating the staphylococcal infection. Puah *et al.* (2016) also reported that tetracycline was the most common antibiotics that are resistant by the *Staph. aureus* isolated from Sushi and Sashimi. Apart from that, a few strains isolated were resistant to teicoplanin, clindamycin, and rifampicin. The antimicrobial resistance could be due to the antibiotic selective pressure in the community. Once oxacillin is given to methicillin-susceptible *Staph. aureus*, it may develop efflux pump against oxacillin. So, the worrying situation is the development of MRSA with teicoplanin-, clindamycin- and rifampicin-resistance.

Extensive use of antimicrobial agents has been the major cause of MDR bacterial development (Bogaard & Stobberingh, 2000). The antimicrobial agents were not only applied in human, veterinary therapeutics and prophylaxis, but also in commercial animals' growth promotion. Majority of the MDR strains isolated in this study were associated with RTE foods. The common ingredients of these RTE foods were chicken, egg and vegetables. Hence, the selective pressure by the antimicrobial agents at the farming level might be the contributory factor. Most of these antibiotic resistance genes were carried by the mobile genetic elements like plasmids, transposons, and integrons.

Eventually human microflora like *E. coli* and *Staph. aureus* could be the excellent reservoir for spreading of MDR bacterial due to horizontal gene transfer.

Besides the dissemination of antimicrobial resistance by RTE foods, horizontal transfer of antimicrobial resistance genes via abiotic surfaces has also raised the public health concern (Kruse & Sørum, 1994; Warnes et al., 2012). Warnes *et al.* (2012) successfully recovered ESBL-producing bacteria from stainless steel surfaces. However, ESBL-producing bacteria remained VBNC on copper and brass surfaces (Warnes et al., 2012).

Despite the fact that most of the studies showed the dissemination of antimicrobial resistance bacteria from poultry products to human or food bacteria, it is also believed that most of the antimicrobial resistance bacteria are widely spread among the community (Johnson et al., 2006) as human gut bacteria could be a reservoir for the dissemination (Szmolka & Nagy, 2013). Liu be an important factor in the transmission of foodborne pathogen. Recognising this aspect in the food chain is important to break the chain of transmission. Proper hand and FCS sanitation could be the best ways to prevent further invasion of potential foodborne pathogens.

Although not many MDR *Staph. aureus* was isolated, prevalence of virulence genes (including enterotoxin genes) was high. Fortunately, no MRSA was isolated. Among the 96.3% of the *Staph. aureus* population isolated that was found harbouring virulence genes, 50% were harbouring at least one staphylococcal enterotoxin (SE) gene, most of them were recovered from food handlers' hands. Udo et al. (2006) reported a higher prevalence of SE genes, 71% in *Staph. aureus* isolated from food handlers' hands while Puah *et al.* (2016) reported 30.8% of the isolated *Staph. aureus* had at least one SE gene. In this study, 29 out of 39 enterotoxigenic St*aph. aureus* carried only one SE gene. However, previous reports are cited on the high prevalence of the co-existence of multiple SE genes in an enterotoxigenic *Staph. aureus* (Cha et al., 2006; Holecková et al., 2002;

Udo et al., 2006). SEA was recognised as the most common SE gene that caused staphylococcal food poisoning (Argudin et al., 2010; Cha et al., 2006; Ghaznavi-Rad et al., 2010) and the third most prevalent SE gene in this study, 11/81, 14%. Enterotoxigenic *Staph. aureus* carried *seg* was the second prevalent SE after *sec* in this study, which was the only non-classical SE that was associated with staphylococcal food poisoning among the new types SEs (Argudin et al., 2010; Ikeda et al., 2005). The prevalence of *sea* and *seh* in this study was slightly higher than the study of *Staph. aureus* from sushi and sashimi by Puah *et al.* (2016) which was 3 (5.8%) and 1 (1.9%), respectively.

Apart from that, *fnb*A was detected in almost all strains (n = 78, 96.3%), followed by *efb* (n = 36, 44%).This finding was further supported by Arciola et al. (2005) and Lim *et al.* (2012) in which *fnb*A was present in almost all *Staph. aureus* isolated while *efb* was present in a high number of *Staph*. aureus strains (Lim et al., 2012). A *Staph*. *aureus* isolated from TCC was found harbouring *pvl*. In the study by Puah *et al.* (2016), pvl-positive *Staph. aureus* was isolated from both sushi and sashimi. The presence of *pvl* was always related to MRSA (Melles et al., 2006), but the *pvl*-positive *Staph. aureus* isolated is not a MRSA. It could be due to possible contamination from a food handler having skin lesion. However, *pvl* was encoded by bacteriophage. Therefore, it could be easily spread to strains in different sources (Jarraud et al., 2002).

All *V. cholerae* isolated was non-O1/O139 and not carrying cholera toxin gene (CTX). Non-O1/O139 *V. cholerae* has been regularly associated with sporadic gastroenteritis in human (Weis et al., 2011). It is gaining public health concern due to the acquisition of virulence genes via mobile genetic elements and caused epidemic cholera. Hasan *et al.* (2015) reported that the non-O1/O139 *V. cholerae* recovered from the gastroenteritis patient carried *hly*A, *tox*R, and *omp*W but not O biosynthesis genes, *ctx*A and *tcp*A, in which the virulotypes was nearly the same as the strains isolated from TCC.

Moreover, *rtx*C was detected in 19 strains isolated from TCC in which responsible for cytotoxic activity on infected human (Lin et al., 1999).

5.3 Dissemination of foodborne pathogens in food premises

The isolated bacteria were genetically diverse, but the strains that were isolated from the same sampling sites were closely-related. Most of the strains have either similar resistotypes or virulotypes within the same cluster. However, there is no evidence of the same resistotypes or virulotypes being clonal in a specific area.

The dendrograms revealed that cross contamination could have occurred via TCCs and food handlers, cutting boards and TCCs and cutting boards and food handlers, indicating poor food handling skill among food handlers (Figure 4.9, Figure 4.11, Figure 4.15 and Figure 4.178). Soon et al. (2011) reviewed that 50% of the food poisoning episodes in Malaysia were caused by improper food handling. This may be attributed to cross contamination and recontamination of RTE foods. Cross contamination will occur when proper sanitation is not followed especially during hand washing and the sanitization of FCS.

Certain food handlers were observed to work in several food premises. These contract workers work at one food premise on one day and at another premise on another day. So the isolates from these food handlers were linked to other isolates from another food premises. The isolates that were from different sampling sites but having 100% similarity (Figure 4.9, Figure 4.15 and Figure 4.17) might be possibly due to this scenario. This scenario illustrates the importance of transmission of potential foodborne pathogens. However, we were not able to see the link of the *E. coli* from food handler, TCC, and FCS with the *E. coli* from foods.

Although 100% similarity between *E. coli* from RTE foods and other sources was not observed, but some *E. coli* isolated from foods were having > 80% similarity with *E.*

coli isolated from other sources. Vincent *et al.* (2010) found that *E. coli* from the patient in his study were indistinguishable or closely related to *E. coli* from food. Hence, Vincent *et al.* (2010) proposed that food could be a reservoir or transmission vehicle for *E. coli* dissemination and eventually causes infection. Moreover, improper sanitation could promote the persistence of *E. coli*.

Cutting board is an important fomite in foodborne pathogen transmission. Wooden cutting boards are porous and suitable habitat for diverse bacteria (Cliver, 2006). The use of wooden cutting boards had long been banned by the Malaysian Ministry of Health. However, these wooden cutting boards are still used by the food handlers in some of the food premises involved in our study. Moreover, some cleaning cloths were observed left on the cutting board after used. Therefore, sanitising cutting board is essential as it has been reported by Cliver (2006) that proper sanitation of cutting boards can effectively diminish the rate of salmonellosis.

Based on the consistency in the banding pattern and the discriminatory power, PFGE was the most suitable method to study the genetic relatedness of the isolated bacteria. More similar resistotypes and virulotypes were observed in the dendrograms. However, not all strains were typeable by PFGE. Therefore, a combination of REP-PCR and PFGE could be better in distinguishing the different sources of the strains.

5.4 Discrepancy between the KAP and microbiological performance of the food handlers

In Malaysia, the food service industry shows an increasing trend of hiring foreign labourers to work as servants, stewards and cooks to prepare foods. The actual number of foreign food workers working in Malaysia is not known because most of them are working on a contract basis. The use of contract workers in food premises has raised the public concern on food operations' ability to ensure food safety (Saad et al., 2013). Our

study here again reflected the scenario where we have more foreign food handlers (61.2%) working in the food premises than the locals (Table 4.7). Other similar studies conducted in another part of Malaysia have also reported a similar scenario (Norrakiah & Siow, 2014; Rosnani et al., 2014; Saad et al., 2013). One of the biggest problems of having more foreign food handlers in Malaysia is the effectiveness of the safe food-handling course which is conducted in either Malay or English language. About a quarter of the food handlers involved in this study (26.9%) received a primary education while the majority (55.2%) were secondary school leavers (Table 4.12). The majority of the foreign food handlers are from India, Pakistan, Nepal and Cambodia in which the English command is low and therefore it is presumed that the safe food-handling course will be of no impact in instilling proper food safety practices among the food handlers. However, our findings did not reflect such a scenario. Although the improvement on knowledge performance was not significant between those who had attended the course and who had not, the safe food handling course had shown a significant impact on instilling positive food safety attitude, particularly on food safety concern among the respondents who were foreigners by majority (Table 4.15). Nonetheless, more detailed work is required to review the effectiveness of the national safe food handling course, particularly to the foreign food workers. An easy-to-understand module such as that based on illustrations could be more efficient in delivering the knowledge to food handlers of different background and education levels.

Of the six constructs assessed on food safety knowledge, the food handlers who participated in this study demonstrated good knowledge on personal hygiene (mean score: 97.7 ± 11.4%) but not on cross-contamination (mean score: 51.1 ± 15.0%). Our finding corroborated with other studies in Malaysia (Norrakiah & Siow, 2014). We believe that this scenario is a reflection of the current safe food handling course in Malaysia which focuses on personal hygiene of food handlers while less emphasis is given to prevention

of cross-contamination. The food handlers demonstrated poor knowledge on foodborne pathogens (19.6 ± 25.1%). This finding is supported by Liu et al. (2012). Based on the study conducted by Saad et al. (2013) on hygiene practices among food handlers in governmental institutions in Malaysia, about 30% of the food handlers commented that the safe food handling course failed to improve their knowledge at work. However, our study suggested that food safety handling course could have significantly improved the awareness of food handlers particularly on foodborne pathogens (Table 4.15). Nevertheless, the finding indicated that the contents of safe food handling course need to be reviewed and improved.

The education level of food handler is generally perceived as one of the factors that compromised the food safety and hygiene. Although we have observed an improvement bin the food safety knowledge among those with tertiary education, But food handlers with lower education level, particularly those who had no formal education could have known more than those with higher education (Table 4.15). For instance, the food handlers without formal education outperformed others on personal hygiene knowledge (Table 4.15). Moreover, it was found that respondents with secondary education demonstrated better attitude concerning food safety practice at work (Table 4.15). These findings are further supported by the Pichler et al. (2014), McIntyre et al. (2013), Lynch *et al.* (2003) and Toh and Birchenough (2000). Working experience, on the other hand, was found to have a significant impact on the overall food safety knowledge among the respondents ($p < 0.05$) in this study: more than 6 years > 4-6 years > 2-4 years > less than or equal to 2 years experience (Table 4.15). Saad et al. (2013) had also reported on the similar observation.

Other studies have reported that provision of food safety and hygiene knowledge is not necessarily translated into safe food behaviour or practice (Ackerley, 1994; Angelillo et al., 2000; Curtis et al., 1993; Pinfold, 1999). Similarly, our results indicated that the

generally moderate performance on food safety questions did not reflect in the microbial hand hygiene assessment. Ironically, food handlers who had *Salmonella*-detected on their hands scored significantly higher (55.4 ± 12.5%) on knowledge related to cross-contamination prevention and sanitation compared to those who had passed the hand hygiene test (46.2 ± 14.9%) (Table 4.15). Interestingly, the high score on self-reported practices observed in this study indicated only the awareness of safe food handling among the participating food handlers, but not always practised in reality. Based on the survey, the respondents who failed the hand hygiene test based on coliforms count (≥ 1.3 log CFU/cm^2) reported that they often wore gloves when they touched or distributed unwrapped food compared to those who passed the hand hygiene assessment where they only frequently but not always wore gloves (Table 4.16). Moreover, our observation did not suggest the frequent usage of gloves during food handling. Overall, the findings suggested that the safe food handling course did, in fact, impart some knowledge and awareness on food safety, but failed to change the safe food behaviour among the food handlers. According to Worsfold et al. (2004), behaviour change in safe food handling could be attained when the knowledge and skills learned are being rehearsed and used. Continual training and management support are important elements in the transfer of knowledge into practice (Seaman & Eves, 2010). In this case, further studies are required to understand the factors that have limited the transfer of knowledge into safe food practice among food handlers.

CHAPTER 6: CONCLUSION

In this study, the majority of samples harboured high levels of contamination from aerobic colony count (ACC). Foods such as salad and *nasi lemak* which contained raw ingredients had higher contamination of ACC, coliforms, and *Salmonella*. Although *Salmonella* DNA was detected in a large number of RTE foods, FCS, TCC, and even on the hands of food handlers by MPN-PCR, no positive *Salmonella* and *V. parahaemolyticus* isolates could be recovered from the culturing method. Overall, the microbiological quality of the cooking environment (FCS and TCC) and the hand sanitation of the food handlers were not satisfactory. Hence, food handlers need to improve their hand sanitation practice and maintain a clean cooking environment.

The majority of the *E. coli* were isolated from RTE foods. All *E. coli* isolates were non-virulent with 33.3% (n = 50) being multiple drug resistant. On the other hand, all *Staph. aureus* isolated harboured at least one virulence gene, but only three strains were MDR. *Salmonella* could not be recovered from the MPN-PCR positive samples. Twenty-six *V. cholerae* isolates were recovered from TCC. No MDR *V. cholerae* was isolated whereas 88.5% (n = 23) contained at least one virulence gene.

Based on both PFGE and REP profiles, *E. coli*, *Staph. aureus* and *V. cholerae* were genetically diverse. The bacteria that was isolated from the same sampling site had almost the same genetic profiles. On the contrary, there were a few bacterial strains isolated from different sampling sites that carries similar genetic profiles. This scenario could be explained by the observation whereby the same food handler worked in several cafeterias within the campus. Furthermore, some bacterial strains isolated from different sources had identical genetic profiles. It could be due to the cross contamination that occurred between TCC, FCS, and food handlers. Thus, safe food handling may not be fully practiced during food preparation. More importantly, the *E. coli* and all *Staph. aureus* isolated were predominantly multiple drug resistant and virulent, respectively.

The food handlers had an adequate knowledge, positive attitudes and good self-reported practices on food safety. Based on the statistical analysis, the education, working experience and attending safe food handling course have improved the food safety knowledge and attitudes of the food handlers significantly. However, the pairwise comparison between the KAP results and microbiological quality assessment demonstrated that the knowledge perceived from the safe food handling course was not translated into their real life food handling practices even though they reported to have good practices in the questionnaire.

The findings of this study suggest that the authority should have a closer monitoring to ensure a clean cooking environment, as well as the proper hand sanitation to prevent cross-contamination. The TCC sampled was highly contaminated with pathogens. Thus, the reusable cleaning cloths could be replaced with disposable wet or dry wipes to decrease cross-contamination and recontamination events. MPN-PCR is a better method for hazard identification compared to the culture-dependent method, as it could quantify and detect the VBNC cells. Future studies should consider this method for hazard identification procedure.

Since the food handlers had poor knowledge on foodborne pathogens and cross contamination prevention, the current safe food handling course should be reviewed to enhance its effectiveness. The KAP assessment solely does not reflect the actual food handling situation while preparing food, hence and the addition of microbiological assessment could provide more information on the real food handling situation.

REFERENCES

Ackerley, L. (1994). Consumer awareness of food hygiene and food poisoning. *Environmental Health, March*, 70-74.

Adams, M. R., & Moss, M. O. (2008). Bacterial agents of foodborne illness- *Stapylococcus aureus food microbiology*. Cambridge: Royal Society of Chemistry.

Alhaj, N., Mariana, N. S., Raha, A. R., & Ishak, Z. (2007). Prevalence of antibiotic resistance among *Escherichia coli* from different sources in Malaysia. *International Journal of Poultry Science, 6*(4), 293-297.

Alvarez, J., Sota, M., Vivanco, A., Perales, I., Cisterna, R., Rementeria, A., & Garaizar, J. (2004). Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. *Journal of Clinical Microbiology, 42*(4), 1734-1738.

Andrews, W. H., & Hammack, T. S. (2003, April 2003). Bacteriological Analytical Manual (BAM). *Food Sampling and Preparation of Sample Homogenate.* Retrieved from http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063335.htm

Andrien, M. (1994). *Social communication in nutrition: A methodology for intervention* Retrieved from http://www.fao.org/docrep/t0807e/t0807e00.htm

Angelillo, I. F., Viggiani, N. M., Rizzo, L., & Bianco, A. (2000). Food handlers and foodborne diseases: knowledge, attitudes, and reported behavior in Italy. *Journal of Food Protection, 63*(3), 381-385.

Appelbaum, P. C. (2007). Microbiology of antibiotic resistance in *Staphylococcus aureus*. *Clinical Infectious Diseases, 45*(Supplement 3), S165-S167.

Arciola, C. R., Campoccia, D., Gamberini, S., Baldassarri, L., & Montanaro, L. (2005). Prevalence of *cna*, *fnb*A and *fnb*B adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant. *FEMS Microbiology Letters, 246*, 81-86.

Argudin, M. A., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins, 2*, 1751-1773.

Arumugaswamy, R. K., Rusul, G., Hamid, S. N. A., & Cheah, C. T. (1995). Prevalence of *Salmonella* in raw and cooked foods in Malaysia. *Food Microbiology, 12*, 3-8.

Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., & Haruki, K. (2003). An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology and Infection, 130*, 33-40.

Asperilla, M. O., Smego, R. A., & Scott, L. K. J. (1990). Quinolone antibiotics in the treatment of *Salmonella* infections. *Review of Infectious Disease, 12*, 873-889.

Balzaretti, C. M., & Marzano, M. A. (2013). Prevention of travel-related foodborne diseases: Microbiological risk assessment of food handlers and ready-to-eat foods in northern Italy airport restaurants *Food Control, 29*(1), 202-207.

Barrabeig, I., Rovira, A., Buesa, J., Bartolomé, R., Pintó, R., Prellezo, H., & Domínguez, À. (2010). Foodborne norovirus outbreak: The role of an asymptomatic food handler. *BMC Infectious Diseases, 10*, 269.

Bloomfield, S. F. (2003). Home hygiene: A risk approach. *Journal of Hygiene and Environmental Health, 206*, 1-8.

Bogaard, A. E. V. D., & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: Links between animals and humans. *International Journal of Antimicrobial Agents, 14*(4), 327-335.

Bou, G., Cerveró, G., Domínguez, M. A., Quereda, C. & Martínez-Beltrán , J. (2000). PCR-based DNA fingerprinting (REP-PCR, AP-PCR) and pulsed-field gel electrophoresis characterization of a nosocomial outbreak caused by imipenem- and meropenem-resistant *Acinetobacter baumanii*. *Clinical Microbiology and Infection*, *6*(12), 635-643.

Brakstad, O. G., Aasbakk, K., & Maeland, J. A. (1992). Detection of *Stapylococcus aureus* by polymerase chain reaction amplification of th *nuc* gene. *Journal of Clinical Microbiology, 30*(7), 1654- 1660.

Busse, M. (1995). Media for *Salmonella*. *International Journal of Food Microbiology, 26*, 117-131.

CDC. (2002). *Staphylococcus aureus resistant to vancomycin*. Retrieved June 10, 2015, from United States: https://www.cdc.gov/hai/organisms/visa_vrsa/visa_vrsa.html

CDC. (2013a). *Antibiotic resistance threats in the United States*. Retrieved May 10, 2015, from Georgia, US: http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

CDC. (2013b, 11 December). Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to ready-to-eat salads (Final Update). *2013 Outbreaks.* Retrieved August 30, 2015, from http://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html

CDC. (2013c). *Surveillance for foodborne disease outbreaks United States, 2013, Annual report*. Retrieved Sept 25, 2015, from Atlanta, Georgia: http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2013-508c.pdf

CDC. (2013d). Typhoid fever. *National Center for Emerging and Zoonotic Infectious Diseases.* Retrieved December 20, 2013, from http://www.cdc.gov/nczved/divisions/dfbmd/diseases/typhoid_fever/

CDC. (2014a, 6 Nov 2014). Cholera- Vibrio cholerae infection. *General information.* Retrieved May 5, 2015, from http://www.cdc.gov/cholera/general/index.html

CDC. (2014b, 17 April 2014). Estimating foodborne illness: An Overview. Retrieved June 20, 2014, from http://wwwn.cdc.gov/foodborneoutbreaks/

CDC. (2014c, 28 April 2014). Trends in foodborne illness in the United States, 2013. *CDC Features.* Retrieved March 26, 2015, from http://www.cdc.gov/features/dsfoodsafetyreport/

CDC. (2015a, 27 May 2015). New CDC data on foodborne disease outbreaks. *Data & Statistics.* Retrieved June 30, 2015, from http://www.cdc.gov/features/foodborne-diseases-data/index.html

CDC. (2015b, 9 March 2015). Technical information. Retrieved October 9, 2015, from http://www.cdc.gov/salmonella/general/technical.html

CDC. (2015c, 25 August 2016). What is Salmonellosis: *Salmonella.* Retrieved September 9, 2016, from http://www.cdc.gov/salmonella/general/index.html

CDC. (2016) PulseNet. Pulsed-field Gel Electrophoresis. Retrieved December 2, 2016, from https://www.cdc.gov/pulsenet/pathogens/pfge.html

Cha, J. O., Lee, J. K., Jung, Y. H., Yoo, J. I., Park, Y. K., Kim, B. S., & Lee, Y. S. (2006). Molecular analysis of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning in South Korea. *Journal of Applied Microbiology, 101*(4), 864-871.

Chambers, H. F. (2001). The changing epidemiology of *Staphylococcus aureus*? *Emerging Infectious Diseases, 7*(2), 178-182.

Chen, C. H., Shimada, T., Elhadi, N., Radu, S. & Nishibuchi M. (2004). Phenotypic and genotypic characteristic and epidemiological significance of ctx^{+} strains of Vibrio *cholerae* isolated from seafood in Malaysia. *Applied Environmental Microbiology*, *70*, 1964-1972

Chitnis, V., Chitnis, D., Verma, S., & Hemvani, N. (1999). Multidrug-resistant *Salmonella typhi* in India. *Lancet, 354*, 514-515.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*(2), 232-260.

Chow, K. H., Ng, T. K., Yuen, K. Y., & Yam, W. C. (2001). Detection of RTX toxin gene in *Vibio cholerae by* PCR. *Journal of Clinical Microbiology, 39*(7), 2594-2597.

Clinical Laboratory Standards Institute. (2015). *M100-S25 Performance standards for susceptibility testing*; Twenty- fifth informational supplement (pp. 1-240). Wayne, USA: CLSI.

Cliver, D. O. (2006). Cutting boards in *Salmonella* cross-contamination. *Journal of AOAC International, 89*(2), 538-542.

Codex Alimentarius Commission. (2001a). *Basic texts- food hygiene- Principles and guidelines for the conduct of microbiological risk assessment*. Rome, Italy: FAO/WHO.

Codex Alimentarius Commission. (2001b). *Basic texts on food hygiene* (2nd Edition ed.). Rome, Italy: Codex Alimentarius.

Collignon, P. (2009). Resistant *Escherichia coli*-- We are what we eat. *Clinical Infectious Diseases, 49*(2), 202-204.

Colque-Navarro, P., Palma, M., Söderquist, B., Flock, J. I., & Möllby, R. (2000). Antibody responses in patients with staphylococcal septicemia against two *Staphylococcus aureus* fibrinogen binding proteins: clumping factor and an extracellular fibrinogen binding protein. *Clinical and Diagnostic Laboratory Immunology, 7*(1), 14-20.

Colwell, R. R. & Grimes, D. J. (2000). *Nonculturable microorganisms in the environment*. ASM Press.

Cormier, R. J., Mallet, M., Chiasson, S., Magnusson, H., & Valdimarsson, G. (2007). Effectiveness and performane of HACCP-based programs. *Food Control, 18*(6), 665-671.

Cunningham, A. E., Rajagopal, R., Lauer, J., & Allwood, P. (2011). Assessment of hygienic quality of surfaces in retail food service establishments based on microbial counts and real-time detection of ATP. *Journal of Food Protection, 74*(4), 686-690.

Curiale, M. (2014). *MPN Calculator (Version Build 23).* Retrieved from http://i2workout.com/mcuriale/mpn/index.html

Curtis, V., & Cairncross, S. (2003). Effect of washing hands with soap on diarrhea risk in the community: a systemic review. *Lancet Infectious Diseases, 3*, 275-281.

Curtis, V., Cousens, S., Mentens, T., Kenki, B., & Diallo, I. (1993). Structured observations of hygiene behaviours in Burkira Faso: validity, variability and utility. *Bulletin of the World health Organisation, 71*(1), 23-32.

Edberg, S. C., Rice, E. W., Karlin, R. J., & Allen, M. J. (2000). *Escherichia coli*: The best biological drinking water indicator for public health protection. *Symposium Series (Society of Applied Microbiology), 29*, 106S-116S.

European Food Safety Authority. (2010). The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008 EFSA Journal, 8, 410, 1496. *EFSA Journal, 8*(1), 1496 [1410pp.].

Eykyn, S. J., & Williams, H. (1987). Treatment of multiresistant *Salmonella typhi* with oral ciprofloxacin. *Lancet, 330*(8572), 1407-1408.

FAO/WHO. (1983). *The role of food safety in health and development.* Report of a Joint FAO/WHO expert committee on food safety, Technical report series 705 WHO.

FDA. (2009, 1 October 2015). FDA Food code 2009. *Chapter 1-Purposes and definitions.* Retrieved November 12, 2015, from http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm186464.htm

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., & Gordon, M. A. (2012). Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet, 379*(9835), 2489-2499.

Finkelstein, R. A. (1996). Cholera, *Vibrio cholerae* O1 and O139, and Other Pathogenic Vibrios. In S. Baron (Ed.), *Medical Microbiology* (4th Ed.). Galveston, Texas, US: The University of Texas Medical Branch at Galveston.

Fraser, J. D., & Proft, T. (2008). The bacterial superantigen and superantigen-like proteins. *Immunology Review, 43*, 225-226.

Fratamico, P. M., & Strobaugh, T. P. (1998). Simultaneous detection of *Salmonella* spp. and *Escherichia coli* O157:H7 by multiplex PCR. *Journal of Industrial Microbiology and Biotechnology, 21*, 92-98.

FSIS. (2011, May 2011). Foodborne illness: What consumers need to know. *Food Safety Information.* Retrieved November 2, 2014, from http://www.fsis.usda.gov/wps/wcm/connect/602fab29-2afd-4037-a75d-593b4b7b57d2/Foodborne_Illness_What_Consumers_Need_to_Know.pdf?MOD=AJPERES

Fukuda, K. (2015). Food safety in globalized world. *Bulletin of the World Health Organization, 93*(4), 209-284.

Garau, J., Xercavins, M., Rodriguez-Carballeira, M., Gomez-Vera, J. R., Coll, I., Vidal, D., . . . Ruiz-Bremon, A. (1999). Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. *Antimicrobial Agents and Chemotherapy, 43*(11), 2736-2741.

Geha, D. J., James, R. U., Cynthia, A. G. & David, H. P. (1994). Multiplex PCR for identification of methicillin resistant Stapylococci in the clinical laboratory. *Journal of Clinical Microbiology*, *32*(7), 1768-1772.

Geser, N., Stephen, R., & Hächler, H. (2012). Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Veterinary Research, 8*(21), 1-9.

Ghaznavi-Rad, E., Shamsudin, M. N., Sekawi, Z., Khoon, L. Y., Aziz, M. N., Hamat, R. A., . . . Neela, V. (2010). Predominance and emergence of clones of hospital-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. *Journal of Clinical Microbiology, 48*(3), 867-872.

Gilbert, R. J., Louvios, J. d., Donovan, T., Little, C., Nye, K., Ribeiro, C. D., . . . Bolton, F. J. (2010). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Communicable Disease and Public Health, 3*(3), 163-167.

Gunn, J. S., Marshall, J. M., Baker, S., Dongol, S. Charles, R. C., & Ryan, T. E. (2014). *Salmonella* chronic carriage:epidemiology, diagnosis and gallbladder persistence. *Trends in Microbiology*, *22*(11), 648-655.

Gurler, Z., Pamuk, S., Yildirim, Y., & Ertas, N. (2015). The microbiological quality of ready-to-eat salads in Turkey: A focus on *Salmonella* spp. and *Listeria monocytogenes*. *International Journal of Food Microbiology, 196*, 79-83.

Gutler, J. B., Doyle, M. P., & Kornacki, J. L. (2017) *Foodborne pathogens: Advantages of virulotyping pathogens over traditional identification and characterization methods*. New York , U.S.A.: Springer.

Hang'ombe, B. M., Sharma, R. N., Skjerve, E., & Tuchili, L. M. (1999). Occurrence of *Salmonella* Enteritidis in pooled table eggs and market-ready chicken carcasses in Zambia. . *Avian Diseases, 43*(3), 597-599.

Hasan, N. A., Rezayat, T., Blatz, P. J., Choi, S. Y., Griffitt, K. J., Rashed, S. M., . . . Grimes, D. J. (2015). Nontoxigenic *Vibrio cholerae* non-O1/O139 isolate from a case of human gastroenteritis in the U.S. Gulf Coast. *Journal of Clinical Microbiology, 53*(1), 9-14.

Holecková, B., Holoda, E., Fotta, M., Kalinácova, V., Gondol', J., & Grolmus, J. (2002). Occurrence of enterotoxigenic *Staphylococcus aureus* in food. *Annals of Agricultural and Environmental Medicine: AAEM, 9*(2), 179-182.

Holmberg, S. D. (1988). Vibrios and *Aeromonas*. *Infectious Disease Clinics of North America, 2*(3), 655-676.

Hooper, D. C. (2002). Fluoroquinolone resistance among Gram-positive cocci. *Lancet Infectious Diseases, 2*, 530-538.

Hooper, D. C., & Wolfson, J. S. (1993). *Mechanism of bacterial resistance to quinolones.* (A. S. f. Microbiology Ed. 2nd ed.). Washington, DC: American Society for Microbiology.

Hoshino, K., Yamasaki, S., Mukhopadhyay, A. K., Chakraborty, S., Basu, A., Bhattacharya, S. K., . . . Takeda, Y. (1998). Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139 *FEMS Immunology and Medical Microbiology, 20*(3), 201-207.

Ikeda, T., Tamate, N., Yamaguchi, K., & Makino, S. (2005). Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H. *Journal of Applied and Environmental Microbiology, 71*(5), 2793-2795.

Ishii, S. & Sadowsky, M. J. (2009). Applications of the rep-PCR DNA fingerinting technique to study microbial diversity, ecology and evolution. *Environmental Microbiology*, *11*(2), 733-740.

IMR. (2014). *National Surveillance of Antibiotic Resistance Report* Retrieved November 20, 2015 from Kuala Lumpur, Malaysia: http://www.imr.gov.my/en/component/content/article/75-english-content/national-collabration/1469-nsar-main.html

Hudzicki J. (2009) Kirby-bauer disk diffusion susceptibility test protocol. *Laboratory protocol* New York, NY: American Society for Microbiology.

Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., . . . Vandenesch, F. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and Human Disease. *Infection and Community, 70*(2), 631-641.

Jevons, M. P. (1961). "Celbenin"-resistant staphylococci. *British Medical Journal, 1*, 124-125.

Jeyaletchumi, P., Tunung, R., Margaret-Selina, P., Chai, L. C., Radu, S., Farinazleen, M. G., . . . Pradeep-Kumar, M. (2012). Assessment of *Listeria monocytogenes* in salad vegetables through kitchen simulation study. *Journal of Tropical Agriculture and Food Science, 40*(1), 55-62.

Johnson, J. R., Kuskowski, M. A., Menard, M., Gajewski, A., Xercavins, M., & Garau, J. (2006). Similarity between human and chicken *Escherichia coli* isolates in the relation to ciprofloxacin resistance status. *The Journal of Infectious Diseases, 194*(1 July), 71-78.

Johnson, J. R., Sannes, M. R., Croy, C., Johnston, B., Clabots, C., Kuskowski, M. A., . . . Belongia, E. A. (2007). Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002-2004. *Emerging Infectious Diseases, 13*(6), 838-846.

Joseph, B., Otta, S. K., Karunasagar, I., & Karunasagar, I. (2001). Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology, 64*(3), 367-372.

Kaliyaperumal, K. (2004). Guideline for conducting a knowledge, attitude and practice (KAP) study. *Community Ophthamology, 4*(1), 7-9.

Kapil, A., Sood, S., Dash, N. R., Das, B. K., & Seth, P. (1999). Ciprofloxacin in typhoid fever. *Lancet, 354*(9173), 164.

Kennedy, K. J., Roberts, J. L., & Collignon, P. J. (2008). *Escherichia coli* bacteraemia in Canberra: incidence and clinical features. *The Medical Journal of Australia, 188*(4), 209-213.

Kimsey, H. H., Nair, G. B., Ghosh, A., & Waldor, M. K. (1998). Diverse CTXϕs and evolution of new pathogenic *Vibrio cholerae*. *Lancet, 352*(9126), 457-458.

Knobben, B. A., Mei, H. C. V. d., Horn, J. R. V., & Busscher, H. J. (2007). Transfer of bacteria between biomaterials surfaces in the operating room -- an experimental study. *Journal of Biomedical Materials Research. Part A, 80*(4), 790-799.

Kong, R. Y. C., Law, W. F., & Wu, R. S. S. (1999). A sensitive and versatile multiplex PCR system for the rapid detection of enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) and enteropathogenic (EPEC) strains of *Escherichia coli*. *Marine Pollution Bulletin, 38*(12), 1207-1215.

Krauss, H., Weber, A., Appel, M., Enders, B., Isenberg, H. D., Schiefer, H. G., . . . Zahner, H. (2003). *Zoonoses Infectious Diseases Transmissible from Animals to Humans* (3rd ed.). Washington, US: ASM press.

Kruse, H., & Sørum, H. (1994). Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied and Environmental Microbiology, 60*(11), 4015-4021.

Kumar, J. D., Negi, Y. K., Gaur, A., & Khanna, D. (2009). Detection of virulence genes in *Staphylococcus aureus* isolated from paper currency. *International Journal of Infectious Diseases, 13*(6), 450-455.

Kusumaningrum, H. D., Riboldi, G., Hazeleger, W. C., & Beumer, R. R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology, 85*(3), 227-236.

Landsheere, G. D. (1983). *Assessment of nutrition-related knowledge, skills and attitudes* B. Schürch (Ed.) *Evaluation of nutrition education in third world communities.* Retrieved from http://orbi.ulg.ac.be/handle/2268/87984

Laupland, K. B., Church, D. L., Vidakovich, J., Mucenski, M., & Pitout, J. D. (2008). Community-onset extended-spectrum b-lactamase (ESBL)–producing *Escherichia coli*: importance of international travel. *The Journal of Infection, 57*(6), 441-448.

Le Loir, Y., Baron, F., & Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Genetic Molecular Research, 2*(1), 63-76.

Lim, K. T., Hanifah, Y. A., Mohd Yusof, M. Y., & Thong, K. L. (2012). Investigation of toxin genes among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Tropical Biomedicine, 29*(2), 212-219.

Lim, K. T., Yeo, C. C., Puthucheary, S., & Thong, K. L. (2009). Characterization of multidrug resistant ESBL-producing *Escherichia coli* solates from hospitals in Malaysia. *Journal of Biomedicine and Biotechnology, 2009*, 1-10.

Lin, W., Fullner, K. J., Clayton, R., Sexton, J. A., Rogers, M. B., Calia, K. E., . . . Mekalanos, J. J. (1999). Identification of a *Vibrio cholerae* RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. *Proceedings of the National Academy of Sciences of the United States of America, 96*(3), 1071-1076.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.-O., Gauduchon, V., . . . Etienne, J. (1999). Involvement of panton-valentine-leukocidin–producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clinical Infectious Diseases, 29*, 1128-1132.

Lindqvist, R., Sylven, S., & Vagsholm, I. (2002). Quantitative microbial risk assessment exemplified by *Staphylococcus aureus* in unripened cheese made from raw milk. *International Journal of Food Microbiology, 78*(1-2), 155-170.

Liu, S., Liu, Z., Zhang, H., Lu, L., Liang, J. & Huang, Q. (2012). Knowledge, attitude and practices of food safety amongst food hanlders in the coasral resort of Guangdong, China. *Food Control*, 47(1), 457-461.

Lynch, R. A., Elledge, B. L., Griffith, C. C., & Boatright, D. T. (2003). A comparison of food safety knowledge among restaurant managers, by source of training and experience, in Oklahoma County, Oklahoma. *Journal of Environmental Health, 66*(2), 9-14.

Macías, Y. F., & Glasauer, P. (2014). *Guidelines for assessing nutrition-related Knowledge, Attitudes and Practices KAP mannual* Retrieved from http://www.fao.org/docrep/019/i3545e/i3545e00.html

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., . . . International Collaboration on Enteric Disease 'Burden of Illness' Studies. (2010). The global burden of nontyphoidal salmonella gastroenteritis. *Clinical Infectious Diseases, 50*(6), 882-889.

Mattick, K., Durham, K., Domingue, G., Jørgensen, F., Sen, M., Schaffner, D. W., & Humphrey, T. (2003). The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food. *International Journal of Food Microbiology, 85*(3), 213-226.

McIntyre, L., Vallaster, L., Wilcott, L., Henderson, S., & Kosatsky, T. (2013). Evaluation of food safety knowledge, attitudes and self-reported hand washing practices in FOODSAFE trained and untrained food handlers in British Columbia, Canada. *Food Control, 30*(1), 150-156.

Melles, D. C., Leeuwen, W. B. v., Boelens, H. A. M., Peeters, J. K., Verbrugh, H. A., & Belkum, A. v. (2006). Panton-Valentine Leukocidin genes in *Staphylococcus aureus*. *Emerging Infectious Diseases, 12*(7), 1174-1175.

Melo, D. B., Menezes, A. P. d. O., Reis, J. N., & Guimarães, A. G. (2015). Antimicrobial resistance and genetic diversity of *Escherichia coli* isolated from humans and foods. *Brazilian Journal of Microbiology, 46*(4), 1165-1170.

Monde, M. d. (2011). *The KAP survey model (knowledge, attitudes and practices).* Retrieved from https://www.spring-nutrition.org/publications/tool-summaries/kap-survey-model-knowledge-attitudes-and-practices

Midelet, G., Kobilinsky, A., & 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*(4), 2313-2321.

Modarressi, S., & Thong, K. L. (2010). Isolation and molecular sub typing of *Salmonella Enterica* from chicken, beef and street foods in Malaysia. *Scientific Research and Essays, 5*(18), 2713-2720.

MOH. (2007). *Annual Report 2007*. Retrieved November 30, 2014, from Malaysia: http://vlib.moh.gov.my/cms/content.jsp?id=com.tms.cms.document.Document_3ee7ae7e-c0a81049-874bd300-f13b84f5

MOH. (2013). *Health facts 2013.* Retrieved October 5, 2015, from http://vlib.moh.gov.my/cms/content.jsp?id=com.tms.cms.section.Section_3549ea8f-c0a81049-874bd300-302e756b

MOH. (2014a). *Health facts 2014*. Retrieved October 5, 2015, from Malaysia: http://vlib.moh.gov.my/cms/content.jsp?id=com.tms.cms.section.Section_3549ea8f-c0a81049-874bd300-302e756b

MOH. (2014b). *The national level 2014 clean, safe and healthy canteen award* [Press release]. Retrieved October 5, 2015, from http://fsq.moh.gov.my/v4/index.php/component/k2/item/769-majlis-anugerah-kantin-bersih-selamat-dan-sihat-peringkat-kebangsaan-2014

MOH. (2015). *Health Facts 2015*. Retrieved June 20, 2016, from Malaysia: http://vlib.moh.gov.my/cms/content.jsp?id=com.tms.cms.document.Document_ef876440-a0188549-82a26f00-e6a36876

Mortlock, M. P., Peters, A. C., & Griffith, C. J. (1999). Food hygiene and hazard analysis critical control point in the United Kingdom food industry: practices, perceptions, and attitudes. *Journal of Food Protection, 62*(7), 786-792.

Nandi, B., Nandy, R. K., Mukhopadhyay, S., Nair, G. B., Shimada, T., & Ghose, A. C. (2000). Rapid method for species-specific identification of *Vibrio cholerae* using a primers targeted to the OmpW. *Journal of Clinical Microbiology, 38*(11), 4145-4151.

Navia, M., Capitano, L., Ruiz, J., Vargas, M., Urassa, H., Schellemberg, D., . . . Vila, J. (1999). Typing and characterization of mechanisms of resistance of *Shigella* spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. *Journal of Clinical Microbiology, 37*(10), 3113-3117.

Noor Izani, N. J., Zulaikha, A. R., Mohammad Noor, M. R., Amri, M. A., & Mahat, N. A. (2012). Contamination of faecal coliforms in ice cubes sampled from food outlets in Kubang Kerian, Kelantan. *Tropical Biomedicine, 29*(1), 71-76.

Noorzaleha, A. S., Rusul, G., Zaiton, H., Abdul, R., Siti, H. I., Nishibuchi, M., & Son, R. (2003). Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. *Food Control, 14*(7), 475-479.

Norrakiah, A. S., & Siow, O. N. (2014). Knowledge, attitudes and practices of food handlers on food safety in food service operations at the Universiti Kebangsaan Malaysia. *Food Control, 37*, 210-217.

Ochiai, R. L., Acosta, C. J., Danovaro-Holliday, M. C., Baiqing, D., Bhattacharya, S. K., Agtini, M. D., . . . Group, D. T. S. (2008). A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bulletin of the World Health Organization, 86*(4), 260-268.

Odonkor, S. T., & Ampofo, J. K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: an interview. *Microbiology Research, 4*(2), 1-11.

Oliveira, M. A. d., Souza, V. M. d., Bergamini, A. M. M., & Martinis, E. C. P. D. (2011). Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control, 22*(8), 1400-1403.

Olsvik, Ø., Wahlberg, J., Peterson, B., Uhlen, M., Popovic, T., Wachsmuth, K., & Fields, P. I. (1993). Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in *Vibrio cholerae* O1 strains. *Journal of Clinical Microbiology, 31*(1), 22-15.

Osaili, T. M., Jamous, D. O. A., Obeidat, B. A., Bawadi, H. A., & Tayyem, R. F. (2013). Food safety knowledge among food workers in restaurants in Jordan. *Food Control, 31*(1), 145-150.

Panisello, P. J., & Quantick, P. C. (2001). Technical barriers to hazard analysis critical control point (HACCP). *Food Control, 12*(3), 165-173.

Paydar, M., The, C. S. J. & Thong, K. L. (2013). Prevalence and characterisation of potentially virulent *Vibrio parahaemolyticus* in seafood in Malaysia using conventional methods, PCR and REP-PCR. *Food Control*, *32*, 13-18.

Perez-Rodriguez, F., Valero, A., Carrasco, E., Garcia, R. M., & Zurera, G. (2008). Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Science and Technology, 19*(3), 131-144.

Pichler, J., Ziegler, J., Aldrian, U., & Allerberger, F. (2014). Evaluating levels of knowledge on food safety among food handlers from restaurants and various catering businesses in Vienna, Austria 2011/2012. *Food Control, 35*(1), 33-40.

Pinfold, J. V. (1999). Analysis of different communication channels for promoting hygiene behavior. *Health Education Research, 14*(5), 629-639.

Puah, S. M., Chua, K. H., & Tan, J. A. M. A. (2016). Virulence factors and antibiotic susceptibility of *Staphylococcus aureus* isolates in ready-to-eat foods: Detection of *S. aureus* contamination and a high prevalence of virulence genes. *International Journal of Environmental Research and Public Health, 13*(2), p(iii). E199.

Pui, C. F., Wong, W. C., Chai, L. C., Nilian, E., Farinazleen, M. G., Cheah, Y. K., . . . Son, R. (2011). Simultaneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits using multiplex PCR. *Food Control, 22*(2), 337-342.

Rammelkamp, C. H., & Maxon, T. (1942). Resistance of Staphylococcus. *Proceedings of Royal Society for Experimental Biology and Medicine, 51*, 386-389.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of Escherichia coli O157:H7 Outbreaks, United States, 1982–2002. *Emerging Infectious Diseases, 11*(4), 603-609.

Reller, B. L., Weinstein, M., Jorgensen, J. H. & Ferraro, M. J. (2009) Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases, 49*(11), 1749-1755.

Rivera, I. N. G., Chun, J., Huq, A., Sack, R. B., & Colwell, R. R. (2001). Genotypes associated with virulence in environmental isolates of *Vibrio cholerae*. *Applied and Environmental Microbiology, 67*(6), 2421-2429.

Rosnani, A. b. H., Son, R., Mohhidin, O., Toh, P. S., & Chai, L. C. (2014). Assessment of knowledge, attitude and practices concerning food safety among restaurant workers in Putrajaya, Malaysia. *Food Science and Quality Management, 32*, 20-27.

Ryan, K. J., & Ray, C. G. (2004). *Sherris medical microbiology* (4th Ed.). New York: McGraw-Hill.

Saad, M., Toh, P. S., & Adil, M. A. M. (2013). Hygiene Practices of Food Handlers at Malaysian Government Institutions Training Centers *Procedia - Social and Behavioral Sciences, 85*, 118-127.

Sáenz, Y., Zarazaga, M., Briñas, L., Lantero, M., Ruiz-Larrea, F., & Torres, C. (2001). Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. *International Journal of Antimicrobial Agents, 18*(4), 353-358.

Sagoo, S. K., Little, C. L., Ward, L., Gillespie, I. A., & Mitchell, R. T. (2003). Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *Journal of Food Protection, 66*(3), 403-409.

Schelin, J., Wallin-Carlquist, N., Cohn, M. T., Lindqvist, R., Barker, G. C., & Rådström, P. (2011). The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence, 2*(6), 580-592.

Seaman, P., & Eves, A. (2010). Perceptions of hygiene training amongst food handlers, managers and training providers—a qualitative study. *Food Control, 21*(7), 1037-1041.

Singh, D. V., Isac, S. R., & Colwell, R. R. (2002). Development of a hexaplex PCR assay for rapid detection of virulence and regulatory genes in *Vibrio cholerae* and *Vibrio mimicus*. *Journal of Clinical Microbiology, 40*(11), 4321-4324.

Sinton, L. (2006). Viable but non-culturable bacteria—menace or myth? *New Zealand Water & Wastes Association Journal, 143*, 31-38.

Slama, K. B., Jouini, A., Sallem, R. B., Somalo, S., Sáenz, Y., Estepa, V., . . . Torres, C. (2010). Prevalence of broad-spectrum cephalosporin-resistant *Escherichia coli* isolates in food samples in Tunisia, and characterization of integrons and antimicrobial resistance mechanisms implicated. *International Journal of Food Microbiology, 137*(2-3), 281-286.

Smith, K., Medus, C., Meyer, S., Boxrud, D., F Leano, & Hedberg, C. (2008). Outbreaks of salmonellosis in Minnesota (1998 through 2006) associated with frozen, microwaveable, breaded, stuffed chicken products. *Journal of Food Protection, 71*(10), 2153-2160.

Sneed, J., Strohbehn, C., Gilmore, S. A., & Mendonca, A. (2004). Microbiological evaluation of foodservice contact surfaces in Iowa assisted-living facilities. *Journal of the American Dietetic Association, 104*(1), 1722-1724.

Soon, J. M., Singh, H., & Baines, R. (2011). Foodborne diseases in Malaysia: A review. *Food Control, 22*(6), 823-830.

Stepanovic, S., Circovik, I. C., Ranin, L., & Svabic-Vlahovic, M. (2004). Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. *Letters in Applied Microbiology, 38*(4), 428-432.

Sun, Y. M., & Ockerman, H. W. (2005). A review of the needs and current applications of hazard analysis and critical point (HACCP) system in food service areas. *Food Control, 16*(4), 325-332.

Szmolka, A., & Nagy, B. (2013). Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. *Frontiers in Microbiology, 4*(258), 1-13.

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerging Infectious Diseases, 18*(5), 741-749.

Tan, S. L., Lee, H. Y., Abu Bakar, F., Abdul Karim, M. S., Rukayadi, Y., & Mahyudin, N. A. (2013). Microbiological quality on food handlers' hands at primary schools in Hulu Langat District, Malaysia. *International Food Research Journal, 20*(5), 2973-2977.

Taulo, S., Wetlesen, A., Abrahamsen, R., G, K., Mkakosya, R., & Grimason, A. (2008). Microbiological hazard idntification and exposure assessment of food prepared and served in rural households of Lungwena, Malawi. *International Journal of Food Microbiology, 125*(2), 111-116.

Teh, C. S. J., Chua, K. H., & Thong, K. L. (2010). Simultaneous differential detection of human pathogenic and non-pathogenic *Vibrio* species using a multiplex PCR based on *gyr*B and *pnt*A genes. *Journal of Applied Microbiology, 108*(1), 1940-1945.

Teh, C. S. J., Thong, K. L., Ngoi, S. T., Ahmad, N., Nair, G. B., & Ramamurthy, T. (2009). Molecular characterization of serogrouping and virulence genes of Malaysian *Vibrio cholerae* isolated from different sources. *The Journal of General and Applied Microbiology, 55*, 419-425.

Teh, C. S. J., Thong, K. L., Osawa, R., & Chua, K. H. (2011). Outbreak-associated *Vibrio cholerae* genotypes with identical pulsotypes, Malaysia, 2009. *Emerging Infectious Diseases, 18*(7), 1177-1179.

Teh, C. S. J., Suhaili, Z. S., Lim, K. T., Khamaruddin, M. A., Yahya, F., Sajili, M. H., Yeo, C. C., & Thong, K. L. (2012). Comparative PCR-based fingerprinting of

Vibrio cholerae isolated in Malaysia. *The Journal of General and Applied Microbiology, 57*(1), 19-26.

Tham, J., Walder, M., Melander, E., & Odenhlt, I. (2012). Prevalence of extended-spectrum beta-lactamase-producing bacteria in food. *Infection and Drug Resistance, 5*, 143-147.

Thoendel, M., Kavanaugh, J. S., Flack, C. E., & Horswill, A. R. (2011). Peptide signaling in the staphylococci. *Chemistry Review, 111*, 117-151.

Thorsteinsdottir, T. R., Haraldsson, G., Fridriksdottir, V., Kristinsson, K. G., & Gunnarsson, E. (2010). Broiler chicken as source of human fluoroquionolone-resistant *Escherichia coli,* Iceland. *Emerging Infectious Diseases, 16*(1), 133-135.

Todd, E. C., Greig, J. D., Batleson, C. A., & Michaels, B. S. (2007). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3. Factors contributing to outbreaks and description of outbreak categories. *Journal of Food Protection, 70*(9), 2199-2217.

Toh, P. S., & Birchenough, A. (2000). Food safety knowledge and attitudes: culture and environment impact on hawkers in Malaysia. Knowledge and attitudes are key attributes of concern in hawker food handling practices and outbreaks of food poisoning and their prevention. *Food Control, 11*(6), 447-452.

Udo, E. E., Al-Mufti, S., & Albert, M. J. (2006). The prevalence of antimicrobial resistance and carriage of virulence genes in *Staphylococcus aureus* isolated from food handlers in Kuwait City restaurants. *BMC Research Notes, 2*(108).

Unnevehr, L. J., & Jensen, H. H. (1999). The economic implications of using a food safety regulatory standard. *Food Policy, 24*, 625-635.

Vecchio, V. G. D., Petroziello, J. M., Gress, M. J., McCleskey, F. K., Melcher, G. P., Crouch, H. K., & Lupski, J. R. (1995). Molecular Genotyping of Methicillin-Resistant *Staphylococcus aureus* via Fluorophore-Enhanced Repetitive-Sequence PCR. *Journal of Clinical Microbiology, 33*(8).

Vincent, C., Boerlin, P., Diagnault, D., Dozois, C. M., Dutil, L., Galanakis, C., . . . Manges, A. R. (2010). Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerging Infectious Diseases, 16*(1).

Wallin-Carlquist, N., Cao, R., Marta, D., da Silva, A. S., Schelin, J., & Radstrom, P. (2010). Acetic acid increases the phageencoded enterotoxin A expression in *Staphylococcus aureus*. *BMC Microbiology, 10*, 147.

Warnes, S. L., Highmore, C. J., & Keevil, C. W. (2012). Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: Implications for public health. *mBio, 3*(6), e00489-00412.

Warren, R. E., Ensor, V. M., O'Neill, P., Butler, V., Taylor, J., Nye, K., . . . Hawkey, P. M. (2008). Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum b-lactamases in the UK. *Journal of Antimicrobial Chemotherapy, 61*(3), 504-508.

Wassenaar, T. M. (2011) *Tracing pathogens in the food chain: Virulotyping of foodborne pathogens*. Cambridge, UK: Woodhead Publishing.

Webber, M., & Piddock, L. J. V. (2001). Quinolone resistance in *Escherichia coli*. *Veterinary Research, 32*(3-4), 275-284.

Weis, K. E., Hammond, R. M., Hutchinson, R., & Blackmore, C. G. (2011). Vibrio illness in Florida, 1998-2007. *Epidemiology and Infection, 139*(4), 591-598.

WHO. (2015a). *Infographics: Estimates of the global burden of foodborne diseases*. Retrieved February 23, 2016, from Geneva, Switzerland: http://www.who.int/foodsafety/areas_work/foodborne-diseases/ferg_infographics/en/

WHO. (2015b). *World Health Day 2015: From farm to plate, make food safe.* Retrieved June 20, 2016, from World Health Organization: http://www.who.int/mediacentre/news/releases/2015/food-safety/en/

Willis, C., Elviss, N., & McLauchlin, J. (2013). *LGR/HPA Co‐ordinated Food Liaison Group Studies: A Follow‐Up Study of Hygiene Practices in Catering Premises at Large Scale Events in the United Kingdom* Retrieved from London, UK: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/325434/Hygiene_when_catering_for_large_events.pdf

Winarno, F. G., & Allain, A. (1990). Street foods in developing countries: learn from Asia. Retrieved from http://www.fao.org/docrep/u3550t/u3550t08.htm#street foods and consumers

Woodford, N., Turton, J. F., & Livermore, D. M. (2011). Multiresistant gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiology Reviews, 35*(5), 736-755.

Worsfold, D., Griffth, C., & Worsfold, P. (2004). A survey of environmental health officers' views of food hygiene training. *British Food Journal, 106*(1), 51-64.

LIST OF PUBLICATION AND PAPERS PRESENTED

Publication

1) H. K., Lee, A. H., Hishamuddin, K. L. Thong & L. C. Chai. (2017). Assessment of food safety knowledge, attitude, self-reported practices, and microbiological hand hygiene of food handlers. *International Journal of Environmental Research and Public Health,*14(55).

Proceedings

- H. K., Lee, K. L., Thong, H., Hassan & L. C. Chai (Dec, 2015). Dissemination of *Escherichia coli* in the Kitchen: The Role of food handlers. Paper presented at International Congress of Malaysian Society for Microbiology 2015, Penang, Malaysia.

- H. K., Lee, L. C., Chai & K. L., Thong (Sept, 2015). Microbial source tracking of *E. coli* in food premises based on repetitive DNA sequences. Paper presented at 22nd Malaysian Society for Molecular Biology & Biotechnology Annual Scientific Meeting, Kuala Lumpur, Malaysia.

- H.K., Lee, L.C., Chai & K.L., Thong (Aug, 2015). Microbial assessment of ready-to-eat foods sold in a tertiary academic institution in Malaysia. Paper presented at 5th International Public Health Conference, Kuala Lumpur, Malaysia.