ANTIBIOTIC RESISTANCE PATTERNS AND GENETIC RELATEDNESS OF *SALMONELLA* SEROTYPES ISOLATED FROM CHICKEN CARCASSES OF RETAIL MARKETS AND SLAUGHTERHOUSES IN KOTA BHARU, KELANTAN

SUHAILY SUHANA BT MOHD YUSOFF

UNIVERSITI MALAYSIA KELANTAN

MASTER OF SCIENCE

2016
Antibiotic Resistance Patterns and Genetic Relatedness of Salmonella Serotypes Isolated from Chicken Carcasses of Retail Markets and Slaughterhouses in Kota Bharu, Kelantan

by

Suhaily Suhana Bt Mohd Yusoff

Thesis submitted in fulfillment of the requirement for the degree of Master of Science

Faculty Veterinary of Medicine

UNIVERSITI MALAYSIA KELANTAN

2016
THESIS DECLARATION

I hereby certify that the work embodied in this thesis is the result of the original research and has not been submitted for a higher degree to any other University or Institution.

OPEN ACCES

I agree that my thesis is to be made immediately available as hardcopy or on-line open acces.

EMBARGOES

I agree that my thesis is to be made available as hardcopy or on-line (full text) for a period approved by the Post Graduate Committee.

Dated from: _______________ Until: _______________

CONFIDENTIAL

(Contains confidential information under the Official Secret Act 1972)*

RESTRICTED

(Contains restricted information as specified by the organization where research was done)*

I acknowledge that Universiti Malaysia Kelantan reserves the right as follows.

1. The thesis is the property of Universiti Malaysia Kelantan
2. The library of Universiti Malaysia Kelantan has the right to make copies for the purpose of research only.
3. The library has the right to make copies of the thesis for academic exchange.

SIGNATURE

SIGNATURE OF SUPERVISOR

IC/PASSPORT NO. NAME OF SUPERVISOR

Date: Date:
ACKNOWLEDGEMENT

Thankful to Allah S.W.T upon completion of my study in Master degree of Science in Microbiology. A highly gratitude to my parent for their encouragements and supports all the time. An appreciation to person who guide and helps a lot during the research conducted, he is Associate Professor Dr. Mohd Mokhtar bin Arshad, my main supervisor. Not to forget, person who help and gave ideas over many molecular works, my co-supervisor, Dr. Erkihun Aklilu Woldergiorgis. Thousands gratitutes to our former dean of faculty, Professor Dr. Mohd Azam Khan bin Goriman Khan for his financial approval over all the research expenses be supported by Faculty Veterinary of Medicine. Acknowledgement to University Sains Malaysia (USM) for skillship training attachment as well to the *Salmonella* website curator, Professor Mark Achtman from University of Warwick in United Kingdom. Lastly, thank you to all my friends from UMK and USM for making the research journey an enjoyable moments, for every moral supports given and helps either directly or indirectly. I wish may Allah make things easy for all of us, gave his blessing and make our life journey to satisfy him and being rewarded with good deeds.

Thank you.
# TABLE OF CONTENT

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>THESIS DECLARATION</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENT</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS &amp; SYMBOLS</td>
<td>xi</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>xii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xiii</td>
</tr>
</tbody>
</table>

## CHAPTER 1 INTRODUCTION

1.1 Overview                                                           | 1    |
1.2 Problem Statement                                                 | 7    |
1.3 Research questions                                                | 7    |
1.4 Hypothesis                                                        | 8    |
1.5 Objectives                                                        | 8    |

## CHAPTER 2 LITERATURE REVIEW

2.1 History of *Salmonella*                                            | 9    |
2.2 *Salmonella*                                                       | 10   |
2.3 Clinical Manifestation of Salmonellosis                            | 12   |
2.3.1 Enteric Fever                                                   | 12   |
2.3.2 Gastroenteritis                                                 | 13   |
2.3.3 Bacteraemia                                                     | 13   |
2.3.4 Extra-Intestinal Focal Infection                                | 14   |
2.3.5 Carrier State

2.4 Host Specificity

2.5 Routes of Transmission
   2.5.1 Vertical Transfer
   2.5.2 Horizontal Transmission

2.6 Mechanism of Antibiotic Resistance
   2.6.1 Penicillins
   2.6.2 β Lactams/ β Lactamase Inhibitors
   2.6.3 Cephalosporin
   2.6.4 Aminoglycosides
   2.6.5 Quinolones
   2.6.6 Tetracycline
   2.6.7 Phenicols
   2.6.8 Trimethoprim and Sulphonamides

2.7 Multi-locus Sequence Typing, MLST

2.8 Why should use MLST?

2.9 Housekeeping Genes
   2.9.1 AroC
   2.9.2 DnaN
   2.9.3 HemD
   2.9.4 HisD
   2.9.5 PurE
   2.9.6 SucA
   2.9.7 ThrA
CHAPTER 3 ANTIBIOTIC-RESISTANCE PATTERNS OF SALMONELLA SEROTYPES ISOLATED FROM CHICKEN

3.1 Introduction 35
3.2 Materials and Methods 36
3.3 Results 38
  3.3.2 Antibiotic-resistance pattern of Salmonella isolates in chicken carcasses 38
  3.3.2 Antibiotic-resistance profile of S.Corrallis, S.Enteritidis, S.Stanley and S.Typhimurium 40
  3.3.3 Resistance pattern for S.Corrallis 43
  3.3.4 Resistance pattern for S.Stanley 45
  3.3.5 Resistance pattern for S.Enteritidis 47
  3.3.6 Resistance pattern for S.Typhimurium 48
3.4 Discussion 49
3.5 Conclusion 57

CHAPTER 4 GENOTYPING OF S.CORVALLIS, S.STANLEY, S.ENTERITIDIS AND S.TYPHIMURIUM ISOLATED FROM CHICKEN CARCASSES

4.1 Introduction 58
4.2 Materials and Methods 60
  4.2.1 Salmonella Isolates 60
  4.2.2 DNA Extraction 61
  4.2.3 Confirmation of Salmonella Genus by Polymerase Chain Reaction (PCR) 61
  4.2.4 Amplification of Seven Housekeeping Genes 62
  4.2.5 PCR for Amplification of OriC and Housekeeping Genes 63
  4.2.6 Gene Fragments and Sequences 64
4.3 Results 65
4.3.1 *Salmonella* genus confirmation using Specific-PCR

4.3.2 PCR for all *Salmonella* Isolates

4.4 PCR bands of 7 MLST Housekeeping Genes for *Salmonella* spp.

4.4.1 PCR Product of *AroC* Gene

4.4.2 PCR Product of *DnaN* Gene

4.4.3 PCR Product of *HemD* Gene

4.4.4 PCR Product of *HisD* Gene

4.4.5 PCR Product of *PurE* Gene

4.4.6 PCR Product of *SucA* Gene

4.4.7 PCR Product of *ThrA* Gene

4.5 Alleles and Sequence Type (STs)

4.6 Genetic Relatedness

4.6.1 Phylogenetic analysis of *S.Corrallis*

4.6.2 Phylogenetic analysis of *S.Enteritidis*

4.6.3 Phylogenetic analysis of *S.Stanley*

4.6.4 Phylogenetic analysis of *S.Typhimurium*

4.7 Cluster Analysis of *Salmonella* isolates

4.8 MLST of *Salmonella enterica* subspecies I

4.9 Discussion

4.10 Conclusion

CHAPTER 5 DISCUSSION

CHAPTER 6 CONCLUSION AND FUTURE WORK

REFERENCES

APPENDIX A

APPENDIX B
<table>
<thead>
<tr>
<th>NO.</th>
<th>Description</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Number of <em>Salmonella</em> serotypes from retail markets and slaughterhouses used in this study.</td>
<td>36</td>
</tr>
<tr>
<td>4.1</td>
<td>Forty of <em>Salmonella</em> isolates from chicken carcasses of retail markets and slaughterhouses used in this study.</td>
<td>60</td>
</tr>
<tr>
<td>4.2</td>
<td>PCR parameters used for identification <em>Salmonella</em> spp. Using P1 and P2 primers.</td>
<td>61</td>
</tr>
<tr>
<td>4.3</td>
<td>List of primers (F : forward, and R : reverse) used for PCR amplification of thrA, purE, sucA, hisD, aroC, hemD and dnaN genes.</td>
<td>62</td>
</tr>
<tr>
<td>4.4</td>
<td>Parameters used for PCR amplification of seven housekeeping genes in <em>Salmonella</em> spp.</td>
<td>63</td>
</tr>
<tr>
<td>4.5</td>
<td>The size of allelic marker within each gene.</td>
<td>69</td>
</tr>
<tr>
<td>4.6</td>
<td>Details of allelic profiles and the sequence types (STs) with the clonal complexes for each <em>Salmonella</em> isolates.</td>
<td>78</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>NO.</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Current scheme for classifying the genus <em>Salmonella</em>.</td>
</tr>
<tr>
<td>3.1</td>
<td>UPGMA dendogram of antibiotic resistance profiles of <em>Salmonella</em> from chicken carcasses from retail markets and slaughterhouses.</td>
</tr>
<tr>
<td>3.2</td>
<td>The simplified antibiotic resistance profiles and trends of 45 strains (exclude ATCC) for <em>Salmonella</em> serotype Corvallis, Enteritidis, Stanley and Typhimurium.</td>
</tr>
<tr>
<td>3.3</td>
<td>UPGMA dendogram for the antimicrobial resistance profiles for <em>S</em>.Corvallis from the two sources.</td>
</tr>
<tr>
<td>3.4</td>
<td>UPGMA of antibiotic resistance profiles for <em>S</em>.Stanley isolates from retail premises and slaughterhouses.</td>
</tr>
<tr>
<td>3.5</td>
<td>UPGMA resistance profiles for <em>S</em>.Enteritidis isolated from retail premises and slaughterhouses.</td>
</tr>
<tr>
<td>3.6</td>
<td>UPGMA of antibiotic resistant profiles for <em>S</em>.Typhimurium from fresh chicken meats at retail premises and slaughterhouses.</td>
</tr>
<tr>
<td>4.1</td>
<td>Specificity of P1 and P2 primer for <em>Salmonella</em> genus.</td>
</tr>
<tr>
<td>4.2(a)</td>
<td>PCR of 24 of <em>Salmonella</em> isolates using P1 and P2 primer.</td>
</tr>
<tr>
<td>4.2(b)</td>
<td>PCR of 18 <em>Salmonella</em> isolates using P1 and P2 primer.</td>
</tr>
<tr>
<td>4.2(c)</td>
<td>PCR of 3 <em>Salmonella</em> isolates using P1 and P2 primer.</td>
</tr>
<tr>
<td>4.3(a)</td>
<td>PCR for <em>AroC</em> genes of 24 <em>Salmonella</em> isolates.</td>
</tr>
<tr>
<td>4.3(b)</td>
<td>PCR for <em>AroC</em> genes of 17 <em>Salmonella</em> isolates.</td>
</tr>
<tr>
<td>4.4(a)</td>
<td>PCR for <em>DnaN</em> genes of 24 <em>Salmonella</em> isolates.</td>
</tr>
<tr>
<td>4.4(b)</td>
<td>PCR for <em>DnaN</em> genes of 17 <em>Salmonella</em> isolates.</td>
</tr>
<tr>
<td>4.5(a)</td>
<td>PCR for <em>HemD</em> genes of 24 <em>Salmonella</em> isolates.</td>
</tr>
<tr>
<td>4.5(b)</td>
<td>PCR for <em>HemD</em> genes of 17 <em>Salmonella</em> isolates.</td>
</tr>
<tr>
<td>4.6(a)</td>
<td>PCR for <em>HisD</em> genes of 24 <em>Salmonella</em> isolates.</td>
</tr>
</tbody>
</table>
4.6(b)  PCR for HisD genes of 17 *Salmonella* isolates. 73

4.7(a)  PCR for PurE genes of 24 *Salmonella* isolates 73

4.7(b)  PCR for PurE genes of 17 *Salmonella* isolates. 74

4.8(a)  PCR for SucA genes of 24 *Salmonella* isolates. 74

4.8(b)  PCR for SucA genes of 17 *Salmonella* isolates. 75

4.9(a)  PCR for ThrA genes of 24 *Salmonella* isolates. 75

4.9(b)  PCR for ThrA genes of 17 *Salmonella* isolates. 76

4.10  Minimal spanning tree (MSTree) of MLST data on 40 of *Salmonella* isolates, isolated from retail premises and slaughterhouses. 81

4.11  Dendogram of genetic relatedness of forty randomly selected *Salmonella* isolates. 87

4.12  UPGMA dendrogram of phylogenetic tree and antibiotic resistance patterns for *S.*Corvallis isolates. 90

4.13  UPGMA dendrogram of phylogenetic tree and antibiotic patterns for *S.*Enteritidis isolates. 92

4.14  UPGMA dendrogram of phylogenetic tree and antibiotic resistance patterns for *S.*Stanley isolates. 94

4.15  UPGMA dendrogram of phylogenetic tree and antibiotic resistance patterns for *S.*Typhimurium. 96
**LIST OF ABBREVIATIONS & SYMBOLS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American Type Control Culture</td>
</tr>
<tr>
<td>BHI</td>
<td>Brain Heart Infusion</td>
</tr>
<tr>
<td>BURST</td>
<td>Based Upon Related Sequence Types</td>
</tr>
<tr>
<td>DLV</td>
<td>Double Locus Variant</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>eBG</td>
<td>eBurstGroup</td>
</tr>
<tr>
<td>MLST</td>
<td>Multilocus Sequence Typing</td>
</tr>
<tr>
<td>MST</td>
<td>Minimum Spanning Tree</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>SLV</td>
<td>Single Locus Variant</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-Borate EDTA</td>
</tr>
<tr>
<td>UPGMA</td>
<td>Unweighted Pair Group Method With Arithmetic Mean</td>
</tr>
<tr>
<td>AMP10</td>
<td>Ampicillin 10μg</td>
</tr>
<tr>
<td>AMC30</td>
<td>Amoxycillin/Clavulanic acid 30μg</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Control Culture</td>
</tr>
<tr>
<td>bp</td>
<td>Base Pair</td>
</tr>
<tr>
<td>CN10</td>
<td>Gentamycin 10μg</td>
</tr>
<tr>
<td>CRO30</td>
<td>Ceftriaxone 30μg</td>
</tr>
<tr>
<td>K30</td>
<td>Kanamycin 30μg</td>
</tr>
<tr>
<td>S10</td>
<td>Streptomycin 10μg</td>
</tr>
<tr>
<td>S3 300</td>
<td>Compound sulphoamides 300μg</td>
</tr>
<tr>
<td>SXT 25</td>
<td>Sulphamethoxazole/Trimethoprim 25μg</td>
</tr>
<tr>
<td>TE 30</td>
<td>Tetracycline 30μg</td>
</tr>
</tbody>
</table>
Corak Rintangan Antibiotik dan Hubungkait Genetik Serotip Salmonella yang Diasingkan Dari Karkas Ayam di Pasar dan Rumah Penyembelihan di Kota Bharu, Kelantan

ABSTRAK

Antibiotic Resistance Patterns and Genetic Relatedness of Salmonella Serotypes Isolated from Chicken Carcasses of Retail Markets and Slaughterhouses in Kota Bharu, Kelantan

ABSTRACT

Non-typhoidal *Salmonella* is one of the most important causes of foodborne illness and chicken products were frequently implicated as the source of the infection. *Salmonella* serotypes had been isolated from chicken carcasses of retail markets and slaughterhouses. In Kelantan, *Salmonella* had been isolated from 64% and 65% of chicken carcasses from retail markets and slaughterhouses in Kota Bharu respectively. However, the relatedness of the *Salmonella* serotypes from the chicken carcasses of the retail markets and slaughterhouses had not been determined. The objectives of the research were to determine the antibiotic-resistance pattern (ARP) and clonal relatedness of *Salmonella* serotypes isolated from chicken carcasses of retail markets and slaughterhouses. *Salmonella* Corvallis, *S.*Enteritidis, *S.*Stanley and *S.*Typhimurium isolated from chicken carcasses of retail markets and slaughterhouses in Kota Bharu were used in this study. The serotypes were tested for their antibiotic susceptibility against 13 antibiotics by using disc diffusion method. Molecular typing of the isolates was conducted by multilocus sequence typing (MLST) of the seven housekeeping genes (*thra*, *purE*, *sucA*, *hisD*, *aroC*, *hemD* and *dnAN*). ARP of *S.*Corvallis from chicken carcasses of retail markets and slaughterhouses were mostly similar. *Salmonella* Stanley and *S.*Typhimurium from slaughterhouses were more resistant to antibiotics compared to those from retail markets. In contrast, *S.*Enteritidis from retail markets were more resistant compared to those from slaughterhouses. Of 45 *Salmonella* isolates tested, 91%, 82%, and 69% were resistant to sulphonamides, tetracycline and streptomycin respectively. Overall, 18% of the isolates were resistant to more than seven antibiotics tested. *Salmonella* Corvallis 4C, 59, 43 and 5C from retail markets, and *S.*Corvallis isolates 64, 13C, 69 and 70 from slaughterhouses belonged to the same sequence type (ST), ST1541. In conclusion, combination of MLST and ARP revealed that *S.*Corvallis from chicken carcasses of retail markets and slaughterhouses in Kota Bharu were clonally related indicating that the serotypes originated from the same sources. The information from this study can be used by relevant authorities to enhance appropriate intervention to reduce *Salmonella* contamination in chicken carcasses at slaughterhouses before the chicken carcasses are distributed to retail markets.
CHAPTER 1

INTRODUCTION

1.1 Overview

Non-typhoidal *Salmonella* causes global health burdens and morbidity. A study by Majowic et al., (2010) reported that every year approximately 93.8 millions cases of *Salmonella* gastroenteritis occurred globally with 155,000 deaths. Of the 93.8 millions cases, 80.3 millions were foodborne. In the United States, non-typhoidal *Salmonella* caused approximately 1.2 million illnesses every year resulted in 23,000 hospitalizations and 450 deaths. The medical cost resulted from the infection was approximately $365 millions annually (CDC, 2013). In Malaysia the prevalence of *Salmonella* food poisoning decreased from 36.61 per 100,000 in 2000 to 14.72 per 100,000 in 2003 (Thong, 2006). Infections or outbreaks of foodborne salmonellosis were frequently reported to be associated with consumption of chicken meat or foods containing chicken meat.

In Thailand in 2009, army reserve force students were hospitalized due to abdominal pain and diarrhea. Epidemiological investigation revealed that consumption of green chicken curry was associated with the illness (odd ratio, 4.5; 95% confidence interval, 0.5 – 42.1) (Sitthi *et al.*, 2012).

In Hong Kong, non-typhoidal *Salmonella* was second pathogen that commonly caused food poisoning outbreak and chicken meats are among the
foods that were associated with the outbreak (HP, 2011). *Salmonella* Typhimurium and *Salmonella* Enteritidis had shown to cause food poisoning associated with consumption of contaminated chicken meat in Egypt (Rabie, *et al*., 2012). In Malaysia, *Salmonella* food poisoning has been reported by the local news. For example, 158 pupils from boarding school in Kuala Nerang, Kedah had food poisoning after they ate „Ayam masak merah” contaminated with *Salmonella* as a result of improper preserved chicken meats. In Sungai Petani, Kedah, food poisoning due to chicken meats caused few deaths with hundreds fell ill. In Terengganu, 5 year-old boy died and sixty people had food poisoning as a result of eating fried chicken contaminated with *Salmonella*. Many initiatives have been recommended to prevent *Salmonella* food poisoning such as improving hygiene during processing, prevention of cross-contamination (Buncic and Sofos, 2012) and adequate cooking (Byelashov and Sofos, 2009). Information on genetic relatedness of *Salmonella* serotypes from different sources and its antimicrobial resistance patterns is also one of the efforts to prevent public health against *Salmonella* infection. This is because, getting to know the genetically related isolates distribution trends is prerequisite for us to give a brief idea on tracing back on where the isolates were originated from and the informations are important to study trend of the *Salmonella* spread occurrence.

In Malaysia, *Salmonella* has been isolated from chickens in the farms. For example, Ong *et al* (2014) reported that of 12,664 samples from poultry farms tested, 11.9% were positive for *Salmonella*. The most common serotypes
were *S. Enteritidis* (3.1%) and *S. Typhimurium* (1.3%). Contamination of chicken meat with *Salmonella spp.* at slaughterhouses has also been reported in Malaysia (Arshad and Che Ibrahim, 2014; Rusul et al., 1996). In Vietnam, a study by Bao et al. (2006) found that of 319 chicken carcasses from 15 abattoirs, 32.8% were *S. Emek*, *S. Hadar* (19.0%), *S. Derby* (8.6%), *S. Typhimurium* (7.8%), and *S. London* (6.9%). In a study also in Malaysia, Arshad and Che Ibrahim (2014) demonstrated that of 20 carcasses from slaughterhouses tested, 13 (65%) were contaminated with *Salmonella spp.* The *Salmonella* serotypes isolated were *S. Corvallis* (61.5%), *S. Enteritidis* (23.1%) and *S. Stanley* (15.4%).

In slaughterhouses, spread of *Salmonella* may occur at various processing stages (Gómez-aldapa *et al.*, 2012; Trampel and Hoffman, 2000; Wotton, 2006). Mixing of the chicken carcasses during scalding with temperature around 50°C and 52°C, which is low enough to kill the pathogens make the cross contamination among the chicken carcasses are unavoidable (Wotton, 2006). De-feathering steps after scalding process produce lots of aerosols during feather removal by rubber fingers on the plucking machine or rotating scrappers also contribute in scattering and dissemination of the bacteria (Gómez-aldapa *et al.*, 2012; Musgrove *et al.*, 1996). Moreover, accumulation of *Salmonella spp.* on the rubber finger of the machine will combine with organic materials that will lead to formation of biofilms. The biofilm formation on the rubber finger also make those carcasses being highly contaminated (Wotton, 2006).
Prevalence of *Salmonella* spp. in chicken meat from retail premises had been reported previously (Arumugaswamy *et al.*, 1995; Freitas *et al.*, 2010). Contamination of chicken meats at retail stores is a public health burden in developed and developing countries such as Russia (Alali *et al.*, 2012), Belgium (Dione *et al.*, 2009), India (Suresh *et al.*, 2011), Egypt (El-Aziz, 2013) and Vietnam (Ta *et al.*, 2014). Previously, in Malaysia, *S.* Kentuckey *S.* Blockley, *S.* Enteritidis, *S.* Chinicol, *S.* Muenchen and *S.* Agona were the dominant serotypes isolated from chicken meats bought from retail premises (Arumugaswamy *et al.*, 1995; Rusul *et al.*, 1996). The prevalence of *S.* Typhimurium, *S.* Corvallis, *S.* Weltevreden and *S.* Enteritidis from chicken carcasses from retail markets were then reported (Arshad *et al.*, 2012; Modarressi and Thong, 2010).

*Salmonella* Typhimurium and *S.* Enteritidis are well known to cause Salmonellosis in human and the most prevalence serotypes found in chicken meats from retails in Turkey (Yildirim *et al.*, 2011), Australia (Fearnley *et al.*, 2011), Egypt (El-Aziz, 2013) and Malaysia (Arshad *et al.*, 2012; Thong and Modarressi, 2010). Retail premises such as wet market, supermarket and roadside stalls are among the popular premises chosen by consumer to buy the source of proteins, such as chicken meats, beef, fishes and others. This is due to the flexibility and convenient circumstances provided by variety of these retail premises. By taking into account such retail premises is a direct medium toward consumers, supplying contaminated chicken meats is a direct hazard to the consumers.
S. Enteritidis, S. Typhimurium, S. Corvallis and S. Stanley have also been reported to be actively associates with food-borne outbreak related to consumption of contaminated chicken meats and this become a public health concern (Archambault et al., 2006; Hendriksen et al., 2011). Isolation of S. Corvallis from chicken meats and food containing chicken products sold at retail premises are common lately and newly emergence serotypes also have frequently isolated from human clinical samples (Cavaco et al., 2007; Modarressi and Thong, 2010; Yoshida et al., 2014). In Thailand, S. Corvallis and S. Stanley were among prevalence serovar isolated from raw chicken meats and human having diarrheal diseases (Bodhidatta et al., 2013). Due to the ubiquitous nature of Salmonella, a typing scheme capable of more detail strain identification is essential for epidemiological studies, because the ability to distinguish these Salmonella isolates is very important to trace the source of infections and outbreaks.

Several methods have been used for deciphering the relatedness among the Salmonella isolates but some have low discriminating power, demanding a considerable amount of expertise, time and quipment. Multilocus sequence typing (MLST) very useful for genetic profiling and also easy to interpret as well for result comparison between laboratories and providing the best phylogenetic relationship inferences. Research by Thong and Modarressi in 2010 from Malaysia has identified presence of multi-drug resistant Salmonella spp. from animal food origin such as raw beef and chicken meats sold at retail premises. They have discovered eleven serovars recovered from 88 Salmonella
isolates (Thong and Modarressi, 2010). Sixty-six of these *Salmonella* isolates shows resistance to tetracycline (73.8%), followed by sulfonamide (63.6%), streptomycin (57.9%), nalidixic acid (44.3%), trimethoprim–sulfamethoxazole (19.3%), ampicillin (17.0%), chloramphenicol (10.2%), cephalotin (8.0%), kanamycin (6.8%), ciprofloxacin (2.2%) gentamycin (2.2%), cefoxitin (2.2%), amoxicillin–clavulanate (1.0%) and amikacin (1.0%). Fifty nine out of 88 isolates (67%) were multi-drug resistant (exhibit resistance toward more than 3 antibiotics). Twenty six of 34 S.Corrvaallis isolates shown highest percentage of resistancy. Meanwhile five isolates of fifteen multidrug resistant S.Typhimurium were resistance to more than eight antibiotics (Thong and Modarressi, 2010).

Study conducted by Donado-Godoy and partners in 2014 regarding prevalence of *Salmonella* serovar and their antimicrobial resistant phenotypes on chicken meats sold from variety retail stores and premises in Colombia also revealed the contamination of the chicken meats with *Salmonella* serovar having multiple drug resistant profiles. A total of 354 of 378 (94%) *Salmonella* isolates were resistant to at least one antibiotics, 133 (35.2%) resistant to five antibiotic, 95 (24.6%) resistant to six to 10 antibiotics and 128 (33.9%) were resistant to 11 to 15 antibiotics (Donado-Godoy et al., 2014).
1.2 Problem Statement

Salmonella infection in humans had been frequently reported to be associated with consumption of chicken meat or food containing chicken meat (Sitthi et al., 2012; Ogata et al., 2009). Salmonella also frequently reported being isolated from chickens in farms, slaughterhouses and retail markets in Malaysia (Ong et al., 2014; Arshad et al., 2014; Arshad et al., 2012; Thong et al., 2010). Thus, contamination of Salmonella in chicken carcasses which were sold at retails such as roadside stall, wet market and supermarket is a direct food poisoning hazard to the consumers. Addition to that, there is no research conducted yet to investigate prevalence of multiple antibiotic resistance as well the genetic relatedness of Salmonella from chicken carcasses of retail premises and slaughterhouses in Kota Bharu, Kelantan.

1.3 Research questions

1. Are the selected Salmonella serotypes isolated from chicken carcasses of retail markets and slaughterhouses in Kota Bharu, Kelantan resistance to multiple antibiotics?

2. Are those selected Salmonella serotypes isolated from chicken carcasses of retail markets and slaughterhouses in Kota Bharu, Kelantan genetically related to each other?
1.4 Hypothesis

1. The selected *Salmonella* serotypes isolated from chicken carcasses of retail markets and slaughterhouses in Kota Bharu, Kelantan resistant to multiple antibiotics.

2. The selected *Salmonella* serotypes isolated from chicken carcasses of retail markets and slaughterhouses in Kota Bharu Kelantan also genetically related to each other.

1.5 Objectives

1. To determine the antibiotic resistance patterns (Antibiograms) of *Salmonella* serotypes isolated from processed chickens from retail markets and slaughterhouses in Kota Bharu.

2. To determine genetic relatedness of the *Salmonella* serotype isolated from the processed chickens of retail markets and slaughterhouses in Kota Bharu, Kelantan using MLST Sequence type (STs) profiles.
CHAPTER 2

LITERATURE REVIEW

2.1 History of *Salmonella*

Karl Joseph Eberth, described the finding of bacillus which was suspected as the main cause of typhoid fever in 1880. Four years later, a pathologist name Georg Theodor August Gaffky confirmed the bacillus and named it Gaffky-Eberth bacillus in 1884 (Rabsch et al., 2013). In 1885, Daniel Elmer Salmon and Theobald Smith (Smith assistant) reported the isolation of bacteria linked to „hog cholera” or „swine fever” and named it *Salmonella* Choleræsuis. The bacteria was similar to *Salmonella serovar* Typhi, where at that time the bacillus were thought could cause enteric disease in humans and farm animals (Grimont, et al., 2000). Name of *Salmonella* are given as an honour to an American veterinary surgeon, Daniel Elmer Salmon (Fabrega and Vila, 2013; Rabsch et al., 2013).

There were three precious findings happened in 1896. Firstly, serum from animals that were immunized exhibit agglutination against typhoid bacillus which isolated from two separate researches by Pfeiffer and Kolle, and also during research by Gruber and Durham. Second, a serum from a typhoid patient that exhibit agglutination against typhoid bacillus during Widal and Grunbaum research also known as Widal serodiagnostic test. Third finding was when two
isolates recovered from patients with clinical symptoms of typhoid but negative upon widal serodiagnostic named „bacille paratyphique” (Grimont et al., 2000).

2.2 Salmonella

*Salmonella* is in the family *Enterobacteriaceae* with phenotypic characteristic of straight rod, generally motile with peritrichous flagella, aerobes and anaerobes. Most of *Salmonella* that belong to subspecies 1 (enterica) can be confirmed with biochemical tests such as the fermentation of glucose, mannitol and dulcitol, inability to ferment sucrose, salicin and lactose, inability to hydrolyse urea, O-nitrophenyl-β-D-galactopyranoside (ONPG)-positive and production of H$_2$S. However, composite media such as triple sugar iron agar (TSI) often be used for *Salmonella* confirmation. The medium contains glucose, lactose and sucrose, an H$_2$S detection system and an indicator also included in the medium. Single colony of isolate can be inoculated to the TSI medium by stabbing into the centre of the butt and continuing down to the base and then streaking the inoculum on to the slope followed with incubation at 37°C within 18-24 hours. Organisms that able to ferment glucose, but not lactose or sucrose, will show an initial acid (yellow) slant in a short period indicates glucose is utilized. Under aerobic condition, TSI slant becomes alkaline (red) because of protein breakdown in the medium where as under anaerobic conditions the butt of the tube, the medium remains acid (yellow), production of hydrogen sulfide, H$_2$S is characterized by a blackening of the medium. (Jones et al., 2000).