CHAPTER 1

GENERAL INTRODUCTION

1.1 *Salmonella* characterization

Salmonella are gram-negative, non-spore-forming, rod-shaped bacilli bacteria. These microorganisms range in diameter from around 0.7 to 1.5 μm, with a length of 2 to 5 μm. The optimal temperature and pH for living of *Salmonella* are 8-45ºC and 6.5-7.5, respectively. They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, predominantly peritrichous motility and are facultative anaerobes, able to surviving with or without oxygen [1]. *Salmonella* belongs to Enterobacteriaceae family. Some *Salmonella* serotypes were reported to be a cause of Salmonellosis in both animal and human [2]. The classification of this genus based on the Kauffmann-White scheme [3], There are of two species, *S*. enterica and *S*. bongori. However, *S*. enterica can be divided into six subspecies: *S*. enterica subsp. enterica (I), *S*. enterica subsp. salamae (II), *S*. enterica subsp. arizonae (IIIa), *S*. enterica subsp. diarizonae (IIIb), *S*. enterica subsp. houtenae (IV), and *S*. enterica subsp. indica (VI). *S*. bongori are rarely isolated from clinical specimens but rather are found mainly in cold-blooded vertebrates and in the environment [4]. More than 2500 serotypes have been identified to date.

1.2 Salmonellosis

Foodborne illness is growing public health problem worldwide [5]. *Salmonella* is an important zoonotic foodborne pathogen which is recognized as a major public health concern worldwide [6-7]. This organism can be transmitted to human by consumer unhygienic condition of farm animal origin-food comprising eggs, milk or any meat. Approximately, 15-20% of cause of human infection is from consuming pork [8]. In general, *Salmonella* multiply in carrier pig's intestine and there are many studies found that pig farms are notorious as the initial point of contamination with *Salmonella* in the

production chain. Slaughtering processes are the critical point to spread the organism from pig guts to carcasses or other pig products [9-12]. The clinical of infected people can be high fever, nausea, vomiting, abdominal cramps severe diarrhoea within 4-48 hours after encounter Salmonella while eating raw meat and undercook pork [13]. Not only infection problem but also problem associated with multidrug resistant Salmonella [14] which is one of major concern in veterinary medicine and public health worldwide. The cause of multidrug resistant Salmonella can be from misuse, overuse of antibiotic in pig farm which makes *Salmonella* develop drug resistance [15-16]. Moreover, the improper temperature in the carcass boiling method and chilling method in Slaughterhouse process are one factor of sub-lethal dose which can increase the ability to drug resistant of *Salmonella* [14],[17]. Furthermore, *Salmonella* can be convinced and transfer antibiotic resistant genes via horizontal gene transfer to other bacteria. Slaughterhouse is the critical point to contaminate and transfer multidrug resistant *Salmonella* from feces to carcass or cross contaminate to any equipments, worker's hand or environment [9].

1.3 Antibiotic resistance

Antibiotic resistance is resistance of bacteria to an antibiotic drug that was effective for treatment of infections caused by it. Bacteria can resistant to antibiotic by innate resistant which is occur in particular bacteria by depending on biology of the bacteria. Moreover, they can resistant to antibiotic by acquire resistant which is comprised of mutation and horizontal gene transfer [18]. The important method to transfer resistance gene is mobile genetic element involved in transferring the resistance genes included integron gene cassettes, transposon or R-plasmid[19-20] which can take part in horizontal gene transfer. Integrons are genetic elements play role in the acquisition and expression of genes conferring antimicrobail resistance [21]. The structure is linear double strand DNA [22]. There are 5' variable region and 3' conservered region. In 5' variable region are comprised of Integrase gene (Int gene), attI and promoter. Integrase is an enzyme to cut out or insert in of gene cassette in attI position and 3' conservered region [21]. In addition, the quaternary compound resistant gene (*QacEΔ1*) and sulfonamide resistant gene (*SulI*) are located at the end of the 3' conserved region.

Integrons can play a role in multidrug resistance as gene cassettes can be inserted into them by integrase [23-24].

1.4 Molecular epidemiology technique

Molecular technique is essential for bacterial typing. It can provide the information to determine about infectious-disease transmission pattern, tracking, surveillance as well as distinguish between strains [25]. There are many tools for *Salmonella* typing such as serotyping, Pulse flied gel electrophoresis (PFGE), multi locus sequence typing (MLST) and whole genome sequencing (WGS). *Salmonella* serotyping is a phenotypic characterization which provided useful epidemiological markers for primary discrimination of *Salmonella* serotypes [26]. However, *Salmonella* serotyping is based on immunological typing and requires a large number of specific sera [27] and provides a lower discriminatory power than other molecular techniques such as Pulsed Field Gel Electrophoresis [28-29] which is based on gel separation of large DNA fragments generated by digestion with a restriction enzyme [30]. However, PFGE has several limitations: the method is technically demanding time-consuming, and require specialist. In addition, data obtained are limited compared to sequence-based methods [31]. Multi locus sequence typing may be more appropriate for investigation of evolutionary and population biology relationships. MLST is a molecular technique based on allelic differences in the nucleotide sequences of the housekeeping genes of various bacterial strains [32]. This method can be used to identify and evaluate interrelationships among *Salmonella* isolates as part of disease surveillance and outbreak investigations [33]. PFGE has been extensively the gold standard [34] MLST is also good for grouping and easily exchanged data between laboratories [33]. Both PFGE and MLST have high discriminatory power [35]. However, Whole genome sequencing (WGS) is greater to more classical typing method than PFGE [34]. It's an advanced next generation sequencing technology that provided access to the massive genetic diversity [36]. It can significantly improve our understanding of bacterial evolution, outbreaks, transmission events and surveillance disease have been shown in a recent studies as well as antimicrobial resistance investigation [37].

Genome-wide association studies (GWAS) is the study of testing large numbers of genetic variants such as single nucleotide polymorphisms (SNPs) or insertions or deletions (indels) or k-mer which associations with interested phenotypes by logistic regression statistically [38-39]. It is an approach that involves rapidly scanning markers across the complete set of DNA or genome to find genetic variation associated with particular trait. Once the genetic associations are identified, it can be used to develop better strategies to detect, treat and prevent the disease. WGS and GWAS are the new tools for epidemiology of veterinary medicine and there isn't any report about them in Thailand. They can be tools for consumer protection and refer to make stand method controlling and protecting of *Salmonella* contamination in swine production chain for exporting.

1.5 Objectives

The objectives of this dissertation were

Chapter 2

- To determine Salmonella serodiversity and antimicrobial resistance profiles of 10 antimicrobial drugs of Salmonella isolated from swine production chain in Chiang Mai and Lamphun, Thailand in 2011-2013.

Chapter 3

- To explore class 1 integrons-carrying *Salmonella* obtained from swine production chains.
- To compare the genetic diversity of sequence types of *Salmonella* spp. recovered from Chiang Mai and Lamphun, Thailand with pulsotypes.

Chapter 4

- To characterize the distribution, diversity and evolution of *S.* enterica circulating in AEC countries

- To compare the spatial and temporal associations of strains obtained from previous study in northern Thailand with strains previously submitted to MLST database (http://mlst.warwick.ac.uk/mlst/ dbs/Senterica) in order to expand existing knowledge of salmonellosis epidemiology in the ASEAN region.

Chapter 5

- To find out genetic diversity of *Salmonella* spp. and antibiotic resistant genes from whole genome sequencing results
- To compare of genetic diversity of WGS result and pulsotypes
- To investigate the survival abilities of *Salmonella* spp. though food production chain.

1.6 The benefit of this study

The effect economic, social and environment

The results of this research can help us to promote the consumers protection from salmonellosis. Understanding of molecular epidemiology in *Salmonella* spp. can increased the ability of controlling, prevention distribution and infection of this pathogen. In addition, this can be helped us to promoted the *Salmonella*-free pork export in the future. The deep understanding of antimicrobial resistance *Salmonella* can enhanced the controlling and prevention of antimicrobial resistance spread from animals to human and environment. Moreover, this study can help to develop the using standard of antimicrobial to decrease the cases due to antimicrobial resistance bacterial infection and death rate.

Technology development

Multilocus sequence typing may be appropriate than PFGE technique for investigation of evolutionary and population biology relationships. MLST database is available online worldwide, so that technique can be provide faster results for disease monitoring and investigation of global epidemiology.

Whole genome sequencing and genome-Wide Association Study are the tools haven't yet to be reported in veterinary medicine. WGS offers greater resolution than MLST typing method and facilitates the study of genetic diversity. Moreover, genome-wide association study is becoming important approach for bacterial gene associated with interested phenotype. This would be the first study in veterinary field in Thailand.