ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA* ISOLATED FROM DOGS AND CATS IN NORTHERN AND NORTHEASTERN THAILAND

Mr. Thanich Chantharothaiphaichit

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Veterinary Public Health
Department of Veterinary Public Health
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2020

การดื้อยาในเชื้อซัลโมเนลลา เอนเทอริกาที่แยกได้จากสุนัขและแมวในภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทย

นายธนิช จันทโรทัยไพจิตร์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA* ISOLATED FROM DOGS AND CATS IN NORTHERN AND NORTHEASTERN THAILAND
By	Mr. Thanich Chantharothaiphaichit
Field of Study	Veterinary Public Health
Thesis Advisor	Professor RUNGTIP CHUANCHUEN, D.V.M., M.Sc., Ph.D.

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.....
Dean of the FACULTY OF VETERINARY SCIENCE

(Professor ROONGROJE THANAWONGNUWECH, D.V.M., M.Sc., Ph.D.)

THESIS COMMITTEE

.....
Chairman

(Professor ALONGKORN AMONSIN, D.V.M., M.Sc., Ph.D.)

.....
Thesis Advisor

(Professor RUNGTIP CHUANCHUEN, D.V.M., M.Sc., Ph.D.)

.....
Examiner

(Dr. TARADON LUANGTONGKUM, D.V.M., M.Sc., Ph.D.)

.....
Examiner

(Dr. SAHARUETAI JEAMSRIPONG, D.V.M., M.P.V.M., Ph.D.)

.....
External Examiner

(Associate Professor Sunpeth Angkittitrakul, D.V.M., M.Sc., Ph.D.)

ธนิช จันทโรทัยไพจิตร์ : การดื้อยาในเชื้อซัลโมเนลลา เอนเทอริกาที่แยกได้จากสุนัขและแมวในภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทย. (ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA* ISOLATED FROM DOGS AND CATS IN NORTHERN AND NORTHEASTERN THAILAND) อ.ที่ปรึกษาหลัก : ศ. สพ.ญ.ดร.รุ่งทิพย์ ชวนชื่น

ปัญหาการดื้อยาต้านจุลชีพของแบคทีเรียถือเป็นหนึ่งในปัญหาสุขภาพหนึ่งเดียว (One Health) ที่เกี่ยวข้องกับมนุษย์ สัตว์ สิ่งแวดล้อมและจำเป็นต้องมีแนวทางเพื่อแก้ไขปัญหาภายใต้แนวคิดสุขภาพหนึ่งเดียว อย่างไรก็ตามข้อมูลของเชื้อดื้อยาในสัตว์เล็กโดยเฉพาะสุนัขและแมวยังคงมีอย่างจำกัด ทั้งสุนัขและแมวถือว่าเป็นสัตว์เลี้ยงที่มีความนิยมอย่างมากและเป็นที่ทราบกันดีว่าสัตว์เลี้ยงเหล่านี้สามารเป็นพาหะของแบคทีเรียก่อโรคหลายชนิดที่มีความสำคัญต่อปัญหาด้านสาธารณสุขรวมถึงเชื้อซัลโมเนลลา (*Salmonella*) ดังนั้นการศึกษานี้มีจุดประสงค์เพื่อจำแนกซีโรวาร์ของเชื้อซัลโมเนลลา เอนเทอริกาและศึกษาลักษณะฟีโนไทป์และจีโนไทป์ของการดื้อยาในเชื้อซัลโมเนลลาที่แยกได้จากสุนัขและแมวในภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทย จำนวนเชื้อทั้งหมด 178 ตัวอย่างจากสุนัข 159 ตัวอย่างและแมว 19 ตัวอย่างที่เก็บรวบรวมระหว่างปี 2558-2561 พบทั้งหมด 77 โดยพบซีโรวาร์ Weltevreden (9.6%), Stockholm (9.0%) และ Typhimurium (7.3%) เป็นส่วนใหญ่ ผลการศึกษาความไวต่อยาต้านจุลชีพพบการดื้อยาหลายขนานถึง 34.3% มีเพียงไอโซเลตเดียวเท่านั้น (ซีโรวาร์ Stockholm) ที่สร้างเอนไซม์ Extended Spectrum Beta- Lactamase (ESBL) และพบยีน bla_{TEM-1} และ $bla_{CTX-M55}$ จากการทดสอบหา plasmid-mediated quinolone resistance (PMQR) genes พบเพียงยีน *qnrS* (10.1%) จากการศึกษาชนิดของพลาสมิดพบพลาสมิดชนิด IncA/C (0.6%), IncN (1.1%) IncFIIA (28.7%), IncHI1 (2.2%), และ IncI1 (3.4%) ชนิด IncFIIA พบมากที่สุด (51 ไอโซเลต, 28.7%) ผลจาก pMLST พบ 5 ST ที่แตกต่างกัน ได้แก่ IncA/C-ST6, IncH1-ST16, IncI1-ST60, IncI1-ST101 และ IncI1-ST136 ซึ่ง ST16 ของพลาสมิด IncHI1 คือพลาสมิด ST ใหม่ สำหรับการศึกษาพลาสมิดชนิด F โดยวิธี Replicon Sequence Typing พบ 4 รูปแบบที่แตกต่างกัน ได้แก่ S1:A-:B- (n=4), S1:A-:B22 (n=2), S3:A-:B- (n=1) และ S-:A-:B47 (n=1) ผลการศึกษาในครั้งนี้ชี้ให้เห็นถึงความสำคัญของสุนัขและแมวในการเป็นพาหะของเชื้อซัลโมเนลลาที่ดื้อต่อยาต้านจุลชีพ ซึ่งเชื้อซัลโมเนลลาเหล่านี้อาจแพร่กระจายสู่มนุษย์และสิ่งแวดล้อมอีกทั้งอาจก่อให้เกิดการดื้อยาในวงกว้าง

สาขาวิชา สัตวแพทยสาธารณสุข ลายมือชื่อนิสิต .....

ปีการศึกษา 2563 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6175305631 : MAJOR VETERINARY PUBLIC HEALTH

KEYWORD: Antimicrobial resistance, Cat, Dog, Plasmid, Salmonella enterica

Thanich Chantharothaiphaichit : ANTIMICROBIAL RESISTANCE IN *SALMONELLA ENTERICA* ISOLATED FROM DOGS AND CATS IN NORTHERN AND NORTHEASTERN THAILAND. Advisor: Prof. RUNGTIP CHUANCHUEN, D.V.M., M.Sc., Ph.D.

Antimicrobial resistance (AMR) is One Health issue involved in humans, animals and environment and a unified One Health approach is required to contain this problematic issue. However, data on AMR in companion animals is limited. Dogs and cats are popular pet animals and are known to carry many bacterial pathogens that are of public health importance, including *Salmonella*. This study aimed to identify serovars of *Salmonella* and phenotypically and genetically characterize AMR in *Salmonella* isolated from dogs and cats in Northern and Northeastern Thailand. A total of 178 *S. enterica* isolates from dogs (n= 159) and cats (n= 19) collected between 2015 – 2018 were included. Seventy-seven serovars were identified, of which serovars Weltevreden (9.6%), Stockholm (9.0%), and Typhimurium (7.3%) were most common. The majority of the isolates (34.3%) were multidrug resistant. Only one ESBL-producing isolate (i.e., serovar Stockholm) was identified and the isolate carried $bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M55}}$. Of all plasmid-mediated quinolone resistance genes tested, only *qnrS* (10.1%) was detected. Plasmid replicon types found were IncA/C (0.6%), N (1.1%) IncFIIA (28.7%), IncHI1 (2.2%), and IncI1 (3.4%). IncFIIA was most prevalent (51 isolates, 28.7%). Based on the pMLST typing scheme, the plasmids were assigned to 5 different STs including IncA/C-ST6, IncH1-ST16, IncI1-ST60, IncI1-ST101, and IncI1-ST136, of which ST 16, of IncHI1 plasmid was the novel plasmid STs. Subtyping F-type plasmids using the RST scheme revealed 4 different combinations of replicons including S1:A-:B- (n=4), S1:A-:B22 (n=2), S3:A-:B- (n=1), and S-:A-:B47 (n=1). The findings highlight the role of household dogs and cats as carriers of AMR *Salmonella enterica* strains with R plasmid and AMR determinants. These *Salmonella* strains may spread to humans and environment and promote the wide distribution of AMR.

Field of Study:	Veterinary Public Health	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....

ACKNOWLEDGEMENTS

First of all, I would like to express my great and unforgettable thanks and appreciation to my advisor, Prof. Dr. Rungtip Chuanchuen, for her precious advice, for her kindness and comprehension and for her support and encouragement throughout the study.

I would like to say thank to my committee chairman, Prof. Dr. Alongkorn Amonsin, for his valuable advice and suggestion. I would also like to show my admiration to committee members, Dr. Taradon Luangtongkum, Dr. Saharuetai Jeamsripong and Asst. Prof. Sunpetch Angkittitrakul for their unforgettable advice and suggestion.

I would like to put my special thanks to my friends and the staff from the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University for their treasured advice, support, and encouragement during the whole study.

Finally, I would like to thank to "The Scholarship from the Graduate School, Chulalongkorn University to commemorate 72nd Anniversary of his Majesty King Bhumibol Adulyadej is gratefully acknowledged and the 90th Anniversary Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund)" for their grant for my research.

Thanich Chantharothaiphaichit

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Pages

LIST OF ABBREVIATIONS

AMR	antimicrobial resistance
Bp	base pair
DNA	deoxyribonucleic acid
DW	distilled water
EDTA	ethylenediaminetetraacetic acid
et al.	at alii, and others
g	gram
M	molar
MDR	multidrug resistance
mg	milligram
MIC	minimum inhibitory concentration
mL	milliliter
N	normality
PCR	polymerase chain reaction
TAE	tris-acetate-EDTA
Tris	tris-hydroxymethyl-aminoethane
µg	microgram
µl	microliter

CHAPTER I INTRODUCTION

Emergence and spread of antimicrobial resistance (AMR) in bacteria has occurred rapidly worldwide. This problematic issue has become a major threat to human and animal health. AMR is currently one of the greatest challenges of global public health, and increasingly spreads to almost all sectors in most parts of the world (Reed et al., 2019). It is evident that a major selective pressure for AMR is antibiotics that have been extensively used in humans, animals and agriculture. This is based on the fact that use of any antimicrobial agents inevitably opens a chance for bacteria to develop resistance that possibly spread to other bacteria intra and interspecies. During the past decades, the prevalence of AMR has dramatically increased to a seriously high level and this phenomenon is mainly driven by over-use and mis-use of antimicrobial agents (WHO, 2019). AMR can occur in human, animal, and environmental sectors, and so considered One Health issue. Therefore, an integrated multi-stakeholder One Health approach is required to contain AMR (Queenan et al., 2016). Currently, a variety of antimicrobials are used for treatment of bacterial infections in humans. At the same time, some of these antimicrobials are used in animals either infection treatment, disease prevention, or growth promotion. The antibiotic sharing is worrisome, since it may interfere the availability of antimicrobials in the near future either in human or animal medicine.

Salmonella is a causative agent of Salmonellosis that is a major foodborne disease in developed and developing countries (EFSA, 2018). AMR has complicated salmonellosis in both humans and animals due to increasing of multidrug resistance (MDR) in *Salmonella*. Infection with MDR *Salmonella* is frequently reported and a particular concern has been raised about resistance to last-line antibiotics (e.g., fluoroquinolones, colistin, new generation cephalosporins, and carbapenems) (Fàbrega and Vila, 2013; Karkey et al., 2018). Despite a self–limited disease, salmonellosis associated with resistance to last-line drugs have been increasingly detected in several countries, resulting in increasing treatment cost and long-term

hospitalization. (Irfan et al., 2015; Anjum et al., 2016). In Thailand, MDR *Salmonella* are commonly isolated from livestock and their food products (Polpakdee et al., 2012; Nuanmuang and Kummasook, 2018). Currently, national AMR surveillance program in food animals and related products has been initiated, of which *Salmonella* is one of target bacterial species has been initiated.

Pets have become an important part of current-modern society, providing companionship to humans especially the elderly, children, and those with special needs. Companion animals, particularly dogs and cats, are considered family members with close relations to their owners. In Thailand, dog and cat population is highest in Central region (1.4 million and 0.86 million, respectively), followed by Northeastern (0.73 million and 0.46 million) and Northern (0.64 million and 0.23 million) (DLD, 2019). Domestic dogs and cats are well known as carries for many bacterial pathogens of public health concern, including *Salmonella* (Guardabassi et al., 2004). *Salmonella* spp. can cause diarrhea in pet animals; however, some pets can be asymptomatic carriers (Hoelzer et al., 2011). Up to date, the considerable number of *Salmonella* serovars have been isolated from domestic dogs and cats (Reimschuessel et al., 2017). This places the animals as one of the important reservoirs of human salmonellosis. Pet animals can be infected with *Salmonella* through their environment and shed the bacteria in their stool. Then, transmission of *Salmonella* from pet animals to humans can occur via ingestion fecal-contaminated food or water and direct contact (Silva et al., 2014). While medically important *Salmonella* serovars for humans (e.g., Enteritidis and Typhimurium) have been isolated from domestic dogs and cats, several studies reported the presence of MDR *Salmonella* strains in animals (Polpakdee et al., 2012; Srisanga et al., 2017). A variety of resistance genes have been identified on conjugative plasmids in *Salmonella* from different sources and geographical locations, indicating that horizontal transfer is a major route of sharing of resistance determinants in the pathogens (Sinwat et al., 2016; Murase et al., 2018). Therefore, the potential for zoonotic spread, and the

emergence of AMR in *Salmonella* spp. among pet animals has been raised (Boerlin and Reid-Smith, 2008).

AMR monitoring/surveillance system is a fundamental tool to detect true causes and estimate root cost of AMR. However, most existing AMR monitoring/surveillance program mainly focus on food animal origin. AMR data from bacteria originated from companion animals remains largely unclear. Despite the established linkage between AMR and food animals, there is less known about the prevalence and characteristics of AMR in bacteria associated with companion animals. As AMR are connected across different sectors, it is difficult to assess the impact of pet animal-related AMR on public health due to limited availability of data. Therefore, we determined the serovars of *Salmonella* from dogs and cats together with their phenotypic and genotypic AMR characteristics to better understand the picture and to follow current situation of AMR in *Salmonella* isolated from companion animals.

Objectives of Study

1. To identify serovars of *Salmonella enterica* isolated from dogs and cats in Northern and Northeastern Thailand.
2. To phenotypically and genetically characterize AMR in *Salmonella enterica* isolated from dogs and cats in Northern and Northeastern Thailand.

Questions of Study

1. What are the serovars of *Salmonella enterica* isolated from dogs and cats in Northern and Northeastern Thailand?
2. What is the antimicrobial resistance phenotype of *Salmonella enterica* isolated from dogs and cats in Northern and Northeastern Thailand?
3. What is the antimicrobial resistance genotype of *Salmonella enterica* isolated from dogs and cats in Northern and Northeastern Thailand?

CHAPTER II LITERATURE REVIEW

1. General characteristics of *S. enterica* and their importance to public health

Salmonella enterica is a Gram-negative bacillus, motile, aerobic to facultatively anaerobic and nonspore-forming bacterium in *Enterobacteriaceae* family, which causing salmonellosis in both humans and animals. Due to considerable geological and dynamic in the prevalence and serovars of *Salmonella* in animals, humans, and environment. The genus of *Salmonella* consists of two species, *S. bongori* and *S. enterica* (Tindall et al., 2005). *S. enterica* included approximately 2,500 serovars and classified by different somatic (O) and flagellar (H) antigen. This pathogen has recognized as one of the major food-borne and important zoonotic pathogens. The economic losses associated with salmonellosis have attracted increasing attention in developed countries in recent years (Balasubramanian et al., 2019).

The severity of salmonellosis depends on *Salmonella* serovars and the host susceptibility, with systemic enteric fever (typhoidal) and localizes in gastrointestinal tract (nontyphoidal) (Osman and Cavet, 2011). Clinical signs of salmonellosis in humans such as fever, nausea, vomiting, diarrhea, and acute septicemia may occur (Dhama et al., 2013) and usually mild. Thus, patients will make a recovery without specific treatment in most cases (Majowicz et al., 2010). However, antimicrobial treatment is required in severe cases with heavy infection.

Additionally, AMR *Salmonella* infections can lead to serious problems, as they can lead to ineffective treatment, and the subsequent spread of AMR (Aarestrup et al., 2007). The transmission of *Salmonella* to humans can occur via ingestion of fecal-contaminated food or water either directly or indirectly (Silva et al., 2014). In recent years *Salmonella* remains the leading cause of food-borne disease among other pathogens throughout the world. Thus, a monitoring program is required to understand the role of zoonotic transmission of *Salmonella* from various sources.

2. *Salmonella* in companion animals

The number of pets has increased dramatically in this modern society, and attention is increasingly devoted to pet welfare. Additionally, companion animals can provide support and friendship to humans, particularly dogs and cats are the most important companion animals in the world and have become a family member with close relations to humans. Close contact between pets and their owners can also be a potential source of *Salmonella* spp. for humans. *Salmonella* has been found in pet dogs and cats worldwide; however, the prevalence differed among regions. Up to date, considerable number of *Salmonella* serotypes have been isolated from domestic dogs and cats (Reimschuessel et al., 2017).

Pet animals can act as an important reservoir of human salmonellosis. Pet animals can become infected with *Salmonella* through their environment, with intermittent shedding the bacteria in their stool. *Salmonella* can cause diarrhea in many pet animals, however carriage of *Salmonella* in dogs and cats may be asymptomatic (Hoelzer et al., 2011). Nevertheless, most of the infections were clinically silent, healthy pets may intermittently shedding *Salmonella* which can infect humans and other animals through the environment, and may also be important in the emergence of AMR among the bacterial population (Lynne et al., 2009). Therefore, pet dogs and cats should be considered as public health concern (Damborg et al., 2016). Children, elderly and immunocompromised persons were a higher risk groups of salmonellosis because they are more susceptible to infection (Hoelzer et al., 2011). Additionally, the main risk factors related to the increase in *Salmonella* in pet dogs and cats could be animal feeding (Lowden et al., 2015). Feeding with raw food has also been related to high prevalence of *Salmonella* (Reimschuessel et al., 2017; Davies et al., 2019). Other factors that have been reported to influence the prevalence of *Salmonella* in pets are the environment where animals live or have possibility to contact with wild animals or other infected animals (Liebana et al., 2002). In contrast, the prevalence of *Salmonella* serotypes isolated in clinical healthy dogs and cats vary notably and may depending on animal behavior in the country (Lowden et al., 2015).

3. Antimicrobial resistance in *S. enterica*

Antimicrobials are being used on many fields including veterinary, livestock, and medical in various purposes, such as therapeutic use to treat many kinds of infectious diseases, growth promoter, and metaphylactic or prophylactic use to prevent from diseases (Barton, 2014). In kind of such practice of using antimicrobials is the main cause to increase AMR in bacteria and may facilitate an occurrence of resistant determinants that can spread to humans, animals, and environment. Use of antimicrobial agents in veterinary fields are one of the factors that increase the emergence of resistance strain not only in livestock, but also in small animals. However, circulation of resistance genes or resistance strains in both humans and animals are also involved a multitude of potential transmission routes, selective pressure of antibiotics and other ecological facilitators as well as horizontal genes transfers (Prestinaci et al., 2015). Transmission of AMR bacteria from animals to humans can occur through a variety of routes, where the ingestion route is probably the most important. AMR has extremely become an issue with salmonellosis in both humans and animals. Increasing AMR in nontyphoid *Salmonella* species was considered as major problem for public health worldwide (Su et al., 2004). Infection with *Salmonella* resistant to multiple drugs in severe cases has been frequently reported and particular concern has been raised to resistance to last-line antibiotics for salmonellosis treatment (Fàbrega and Vila, 2013; Karkey et al., 2018). One of a high rate of resistance was shown in *S.* Typhimurium, MDR *S.* Typhimurium DT 104 emerged during the past as a global health problem (Helms et al., 2005). Because MDR *Salmonella* serotypes has been increased, conventional antibiotics have failed to treat. Cephalosporins and fluoroquinolones have become the drugs of choice for the treatment of infection. However, many countries have been reporting outbreaks of infections due to *Salmonella* that were resistant to fluoroquinolones and high generation cephalosporins (Liakopoulos et al., 2016). Recently, resistance rates to critically important antimicrobial agents, e.g., third generation cephalosporins and fluoroquinolones are significantly increased worldwide due to the production of Extended-Spectrum β-lactamase (ESBL) and mutation in quinolone resistance-determining regions (QRDRs) (Liakopoulos et al., 2016; Michael and Schwarz, 2016).

4. Genetics of antimicrobial resistance

4.1 Extended Spectrum β-Lactamases (ESBL)

The primary mechanism of resistance to β-lactam drugs is the production of β-lactamases that hydrolyze the β-lactam ring of the drugs. ESBL is an β-lactamase enzyme, the effect of this enzyme causes the resistance to various types of β-lactam antibiotics, including penicillins, cephalosporins, and aztreonam. However, the ESBL-producing strains are still not possible to resistance to cephamycin (e.g., cefoxitin and cefotetan) and carbapenem (e.g., Imipenem, Ertapenem, and Meropenem) antibiotics and may able to inhibit by β-lactamase inhibitors such as clavulanic acid sulbactam or tazobactam (Tooke et al., 2019). Most of bacteria in *Enterobacteriaceae* can produce ESBLs and make these drugs ineffective for treating infections. In these cases, mean that there are fewer antibiotic options available to treat ESBL-producing *Enterobacteriaceae* infections.

The spread of ESBL is commonly transmitted via transferable elements carried by antibiotic-resistant plasmids, integrons, and transposons (Lindblom et al., 2019). β-lactamases were classified by molecular & functional, which generally consists of Temoniera (TEM), Sulfhydryl variable (SHV), and CTX-M types (Palzkill, 2018). In particular, plasmids that can transmit ESBLs genes and are frequently found to carry other resistance genes, such as fluoroquinolone or chloramphenicol. If so, using a single drug can co-select for several resistance genes resulting in MDR phenotype (Cordeiro et al., 2016).

The majority of ESBLs in *Salmonella enterica* are derivatives of the TEM, SHV, and CTX-M β-lactamase families that can lead to multidrug resistance in *Salmonella enterica* isolates (Pokharel et al., 2006; Ahamed Riyaaz et al., 2018). In addition, there had an evidence of horizontal transmission of bla_{CTX-M} among bacterial species via transferable plasmids between *Salmonella* strains worldwide (Weill et al., 2004; Kim et al., 2016). ESBL-producing *Salmonella enterica* are mostly reported from livestock animals and still have a tendency to be increased (EFSA, 2019). In recent years, ESBL-producing *Enterobacteriaceae* colonization in food animals along with companion animals is constantly increasing (Doi et al., 2017). In Thailand ESBL-producing *Salmonella* have also been reported in humans and food animals (Sinwat et al.,

2016; Trongjit et al., 2017). However, data on *Salmonella* associated to ESBL production in companion animals has only a few reports (Srisanga et al., 2017).

4.2 Quinolone resistance

Quinolone resistance is generally associated with chromosomal mutations in DNA gyrase and/or topoisomerase IV (Quinolone Resistance Determining Region; QRDR) Moreover, plasmid-mediated quinolone resistance (PMQR) genes was also shown to confer resistance and reduce the susceptibility to quinolone drugs (Hooper and Jacoby, 2015) and has increasingly emerged worldwide (Stephenson et al., 2009).

4.3 Quinolone Resistance Determining Region (QRDR)

Chromosomal mutations in DNA gyrase and/or topoisomerase II (GyrA and GyrB) and IV (ParC and ParE), which are involved in bacterial DNA replication. DNA gyrase composed of 2 pairs of subunits including GyrA and GryB encoded by the *gyrA* gene, and *gryB* gene, respectively. Topoisomerase IV composed of ParC and ParE subunits encoded by *parC* gene and *parE* gene, respectively. These chromosomal mutations altered the target enzymes and reduced drug-binding affinity (Jacoby, 2005). In general, the occurrence of two-point mutations in *gyrA* caused high resistance rate or reduce susceptibility to fluoroquinolone more than one-point mutation. Mutations in different locations of both *gyrA* and *parC* caused different levels of resistance. And mutations in both *gyrA* and *parC* resulted in higher levels of resistance to fluoroquinolone, such as *gyrA* mutation combination with *parC* and/or *parE* mutations have shown the higher levels of resistance (Ling et al., 2003).

4.4 Plasmid-mediated quinolone resistance (PMQR)

PMQR has been shown to confer resistance of quinolone and can be transmitted by horizontal gene transfer, which often found in *Enterobacteriaceae* (Carattoli, 2013). Three families of genes are related to PMQR including the target protection protein encoded by *qnr*, the enzymatic modification gene encoded by *aac (6')-Ib-cr* (aminoglycoside acetyltransferase) and the important role in efflux pump encoded by *qepA* (Robicsek et al., 2006). Several studies found that PMQR can

reduce the effects of fluoroquinolones by increased MIC values. Therefore, it is necessary to monitoring the spread of PMQR (Fang, 2015).

In recent years, prevalent of fluoroquinolone resistance in *Salmonella* has been increasingly reported worldwide. In *Salmonella*, quinolone resistance is mainly mediated by point mutations in the QRDR, and can also be acquired by a number of PMQR genes, including the *qnr*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB* (Lin et al., 2015). In addition, the combination between QRDR and PMQR have been shown the higher levels of resistance especially, in high level of expression of *oqxAB, aac(6′)-Ib-cr* and mutation in *gryA* which can lead to reduce susceptibility to fluoroquinolones (Miró et al., 2004).

4.5 Colistin resistance

Colistin is a broad-spectrum antimicrobial drug with belongs to polymixins groups. This drug was used as a last-line drug for dealing with infections caused by wide drug-resistant Gram-negative bacteria including most species of the family *Enterobacteriaceae*. The resistance mechanism to polymyxins is lipopolysaccharide modification of the bacterial outer membrane, resulting in reduction of polymyxin affinity that associated with chromosomal mutations and plasmid-borne *mcr* genes (Liu et al., 2016; Xu et al., 2018). This drug has been widely used in animal production in most of countries for therapeutic, prophylactic and growth promotion purposes. The prolong used lead to a selection of resistant strains that can be spread by direct contact of animal-to-human or indirectly along the food chain. The dissemination of resistance determinants is facilitated by gene transfer mechanisms, such as conjugation (Yutin, 2013). Recently, plasmid-mediated colistin resistance was characterized worldwide. Until now there were nine variants of the *mcr* gene, *mcr-1* – *mcr-9*, have been identified in *Enterobacteriaceae* (Carroll et al., 2019).

S. enterica strains have developed resistance to many classes of antibiotics. Plasmid-mediated colistin resistance conferred by *mcr* genes have been identified in different serovars of *S. enterica strains and* have been detected in isolates from different sources, such as food products, human samples, and food-producing animals (Liu et al., 2016; Garcia-Graells et al., 2018). In China, the investigation found

mcr genes in *Enterobacteriaceae* isolated from dogs and cats representation that the *mcr*-positive pathogens can be transmitted between companion animals and humans (Zhang et al., 2016; Wang et al., 2018). However, in Thailand plasmid-mediated colistin resistance has rarely been reported in companion animals.

5. Transfer of AMR determinants and role of plasmid in AMR distribution

The emergence and spread of AMR among pathogenic bacteria are considered as the public health problem. AMR bacteria has been constantly developing and horizontal gene transfer through plasmids plays a major role (Rozwandowicz et al., 2018). Major pathways of transfer resistance determinants in pathogenic bacteria consist of (I) vertical transfer of resistant bacteria occurs in a clonal spread of individual resistance strain, and (II) horizontal transmission of AMR genes (Von Wintersdorff et al., 2016). Mechanisms of horizontal gene transfer including conjugation is requiring cell to cell contact, which transfers DNA from donor cell to recipient cell; transformation, is the uptake naked DNA fragments from extracellular and transduction, bacteriophages may transfer bacterial DNA from previously infected donor cell to other cells (Devanga Ragupathi et al., 2019)

Plasmids are extra-chromosomal circular of DNA that can autonomously replicate in a host cell. Distribution of AMR determinants in *Enterobacteriaceae* were highly associated with mobile genetic elements (MGEs) and could potentially transfer the resistance determinants through horizontal gene transfer via conjugative plasmid (Szmolka and Nagy, 2013; Chen et al., 2016). Moreover, plasmids are providing additional functions to their bacterial host cell such as AMR, especially R plasmid; a plasmid that carries resistant determinants for resistance to antibiotics. It offers an advantage to their host cells under the antimicrobial pressure. Plasmid is importance because it can carry several resistance genes can therefore result in acquisition of novel resistance genes by pathogenic bacteria and cause the spread of AMR (Thomas and Nielsen, 2005).

The recent assessment of genetic stability of plasmids along with the recurrent identification of resistance plasmids in clinical isolates of several food-borne pathogens suggest plasmids as an important reservoir of AMR genes that can

be effortlessly propagated over the time and across species (Stokes and Gillings, 2011; Sultan et al., 2018). A co-located of resistance and virulence genes on same plasmid was identified in *S.* Typhimurium (Jain et al., 2018). In this case, a single drug selects for both resistance and virulence genes, resulting in a serious issue for public health.

6. Plasmid characterization technique

6.1 PCR-based replicon typing

Plasmids were classified by incompatibility (Inc) groups, defined as the inability of two plasmids to coexist stably over a number of generations in the same bacterial cell (Novick, 1987). Nowadays, PCR-based replicon typing now available to classify and identify the Inc groups of plasmid family in *Enterobacteriaceae* (Carattoli et al., 2005). This method is based on 5 multiplex and 3 simplex PCR and was conducted to detect 18 replicon types. Identification and characterization of plasmid can provide the comprehension of the contribution of plasmid in the dissemination of AMR determinants (Rozwandowicz et al., 2018).

6.2 Plasmid multilocus sequence typing

The plasmid multilocus sequence typing (pMLST) is a supplementary tool for classify the plasmids, which are in the same Inc group and to categorize the plasmid in each Inc group into sequence types on basis of different DNA sequence at the specific loci of each plasmid, and this scheme is used to analyze the different sequence types by selecting multiple target genes (Carattoli et al., 2014). Presently, pMLST was developed to identify and subtype plasmid IncF, I1, N, HI1, HI2 and A/C (García-Fernández et al., 2008; Phan et al., 2009; Villa et al., 2010; Hancock et al., 2017). Characterization of plasmid Inc groups has been used as tool for epidemiological study to monitor the circulation of plasmids among bacterial strains of different sources or to track the horizontal transmission of AMR genes among the *Enterobacteriaceae* (Carattoli et al., 2005).

CHAPTER III MATERIALS AND METHODS

This study comprises 3 phases, including phase 1: Confirmation and serotyping *Salmonella* isolated from dogs and cats in Northern and Northeastern Thailand, phase 2: Detection of AMR phenotype in *Salmonella*, and phase 3: Genetic characterization of AMR in *Salmonella* (Figure1).

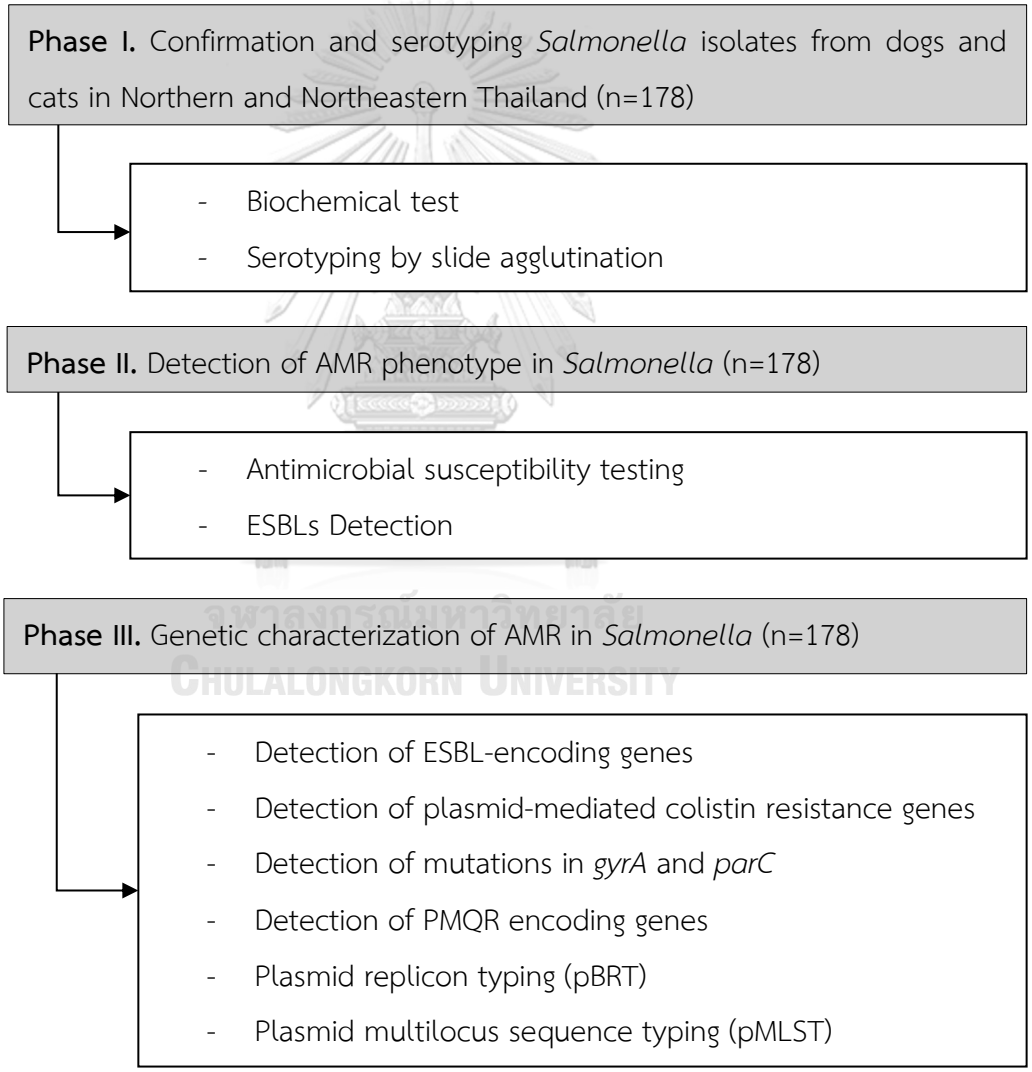


Figure 1 : Experimental design of this study

Phase 1: Confirmation and serotyping *Salmonella* isolated from dogs and cats in Northern and Northeastern Thailand (n=178)

1. *Salmonella* isolates

One hundred seventy-eight of *Salmonella* strains isolates were obtained from the bacterial collection of the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand (Table 1). All *Salmonella* isolates were originated from clinically healthy dogs and cats in 5 Northern provinces (i.e., Chiang Mai, Chiang Rai, Lampang, Mae Hong Son and Nan) and 7 Northeastern provinces (i.e., Bueng Kan, Loei, Mukdahan, Nakhon Phanom, Nakhon Ratchasima, Nong Khai and Roi Et) during 2015 - 2018. All were isolated from fecal samples of household dogs and cats. The animals exhibited a normal health status. However, details on their antibiotic exposure were not revealed.

The *Salmonella* isolation was performed by using a standard method according to the International Organization for Standardization (ISO) standard 6579-1:2002 (ISO, 2002). One isolate was collected from each positive sample and stored in 20% glycerol at -80°C.

2. Determination of *Salmonella* serovars

Serovars of all *Salmonella* isolates were determined by slide agglutination test based on the Kaufman–White scheme (Grimont and Weill, 2007) using a commercially available antiserum (ECDC 2012; S & A Reagents Lab, Bangkok, Thailand).

Table 2 : Source and number of *Salmonella* included in this study (n=178)

Region	**No. of *Salmonella* isolates**	
	Dogs (n=159)	**Cats (n=19)**
Northern	29	7
North-Eastern	130	12
Total	178	

Phase 2: Detection of AMR phenotype in *Salmonella* (n=178)

1. Antimicrobial susceptibility testing (n=178)

All *Salmonella* isolates were examined for antimicrobial susceptibilities by determination of Minimum Inhibitory Concentrations (MICs) using two-fold agar dilution technique according to the Clinical and Laboratory Standards Institute protocol (CLSI, 2013). Broth microdilution technique was carried out to detect colistin susceptibility. The antimicrobial agents tested (clinical breakpoints in parentheses): included ampicillin (32 µg/mL), ceftriaxone (4 µg/mL), chloramphenicol (32 µg/mL), ciprofloxacin (4 µg/mL), colistin (2 µg/mL), levofloxacin (8 µg/mL), gentamicin (8 µg/mL), streptomycin (32 µg/mL), sulfamethoxazole (512 µg/mL), tetracycline (16 µg/mL), and trimethoprim (16 µg/mL). All antimicrobial agents were purchased from Sigma-Aldrich® Chemicals Company (St. Louis, MO). The strains exhibiting resistance to three or more different classes of antimicrobials were considered multidrug resistance (MDR) (Magiorakos et al., 2012).

Detection of carbapenem (meropenem and imipenem) resistance phenotype was performed in all *Salmonella* isolates. Susceptibility to meropenem and imipenem were determined by using disk diffusion method (CLSI, 2014). The *Salmonella* isolates exhibiting resistance to meropenem were phenotypically confirmed by modified Hodge test (MHT) with meropenem (10 µg). The MHT positive-*Salmonella* isolate was considered as carbapenem resistance. All antimicrobial disks were purchased from Oxoid Limited (Oxoid®, Hamshire, England, United Kingdom). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains.

Detail of antimicrobial agents tested is shown in Table 2.

Table 3 : Solvent, range of concentration and breakpoint of antimicrobials test

Antimicrobials	Solvent	Method	Concentration range (µg/mL)	Clinical breakpoint (µg/mL)
Ampicillin	Distilled water	Agar dilution	0.25 – 1,024	32
Ceftriaxone	Distilled water	Agar dilution	0.015625 – 8	4
Chloramphenicol	95% ethanol	Agar dilution	1 – 512	32
Ciprofloxacin	0.1 N NaOH	Agar dilution	0.015625 – 64	4
Colistin	Distilled water	Broth microdilution	0.25 - 64	2
Gentamicin	Distilled water	Agar dilution	0.0125 – 64	8
Levofloxacin	Acetic acid	Agar dilution	0.0625 – 64	8
Streptomycin	Distilled water	Agar dilution	1 – 1,024	32
Sulfamethoxazole	1 N NaOH	Agar dilution	2 – 2,048	512
Tetracycline	70% ethanol	Agar dilution	0.0625 - 256	16
Trimethoprim	Dimethylacetamide	Agar dilution	0.25 – 1,024	16

2. Detection of ESBL production (n=178)

All *Salmonella* isolates were tested for ESBL production using disk diffusion method (CLSI, 2014). Initial screening of ESBL production was performed using 3 indicator cephalosporins, including ceftazidime (30 µg), cefotaxime (30 µg) and cefpodoxime (10 µg). All isolates showing resistance to at least one of the indicator cephalosporin antibiotics were subjected to ESBL-phenotypic confirmation using a combination disk assay. Antibiotic disks used were cefotaxime and cefotaxime (30 mg)/clavulanic acid (10mg) disks and ceftazidime and ceftazidime (30 mg)/clavulanic acid (10 mg). All antimicrobial disks were purchased from Oxoid Limited (Oxoid®). A difference of ≥5 mm between the inhibition zone of the cephalosporin/clavulanic acid combination and corresponding cephalosporin alone disks were considered ESBL positive strains. *Klebsiella pneumoniae* ATCC 700603 served as quality control strains.

Phase 3: Genetic characterization of AMR in *Salmonella* (n=178)

DNA templates for PCR from all *Salmonella* isolates (n=178) were prepared using whole cell boiled lysate (Lévesque et al., 1995). Briefly, single colonies were suspended in 100 µl of sterile distilled water heated in boiling water for 10 min and centrifuged at 12,000 rpm for 5 min. The supernatant was removed to new sterile micro-centrifuge tube and stored at -20ºC for the DNA template for PCR. All PCR reactions were conducted using Toptaq PCR Master Mix Kit (GeNeiMasterMix; Merck, Munich, Germany) according to the manufacturer's instructions. PCR primers are listed in Table 3.

All PCR products were separated using electrophoresis on 1.5% agarose gel (Vivantis®, Subang Jaya, Malaysia) in 1X Tris-acetate/EDTA (1X TAE) buffer. The gel was stained with Redsafe™ Nucleic Acid Staining Solution (iNtRon Biotechnology®, Seongnam, South Korea). Visualization of PCR products was carried out under UV light using Bio-Rad Gel-Documentation System (Bio-Rad Laboratories, California, USA).

Table 4 : Primers used in this study

Target	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
ESBLs				
bla_{CTX-M}	blaCTX-M-F	CGATGTGCAGTACCAGTAA	585	(Batchelor et al., 2005)
	blaCTX-M-R	AGTGACCAGAATCAGCGG		
bla_{TEM}	blaTEM-F	GCGGAACCCCTATTT	964	(Hasman et al., 2005)
	blaTEM-R	TCTAAAGTATATATGAGTA AACTTGGTCT		
bla_{SHV}	blaSHV-F	TTCGCCTGTGTATTATCTCCCTG	854	(Hasman et al., 2005)
	blaSHV-R	TTAGCGTTGCCAGTGYTG		
PMCR				
mcr-1	mcr-1F	AGTCCGTTTGTTCTTGTGGC	320	(Rebelo et al., 2018)
	mcr-1R	AGATCCTTGGTCTCGGCTTG		
mcr-2	mcr-2F	CAAGTGTGTTGGTCGCAGTT	715	(Rebelo et al., 2018)
	mcr-2R	TCTAGCCCGACAAGCATACC		
mcr-3	mcr-3F	AAATAAAAATTGTTCCGCTTATG	929	(Rebelo et al., 2018)
	mcr-3R	AATGGAGATCCCCGTTTTT		

Table 3. (continue)

Target	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
mcr-4	mcr-4F	TCACTTTCATCACTGCGTTG	1,116	(Rebelo et al., 2018)
	mcr-4R	TTGGTCCATGACTACCAATG		
mcr-5	mcr-5F	ATGCGGTTGTCTGCATTTATC	1,644	(Rebelo et al., 2018)
	mcr-5R	TCATTGTGGTTGTCCTTTTCTG		
QRDR				
gryA	gryA-F	GCTGAAGAGCTCCTATCTGG	436	(Chuanchuen and Padungtod, 2009)
	gryA-R	GGTCGGCATGACGTCCGG		
parC	parC-F	GTACGTGATCATGGATCGTG	390	(Chuanchuen and Padungtod, 2009)
	parC-R	TTCCTGCATGGTGCCGTCG		
PMQR				
qnrA	qnrA-F	ATTTCTCACGCCAGGATTTG	516	(Stephenson et al., 2009)
	qnrA-R	GATCGGCAAAGGTTAGGTCA		
qnrB	qnrB-F	GATCGTGAAAGCCAGAAAGG	469	(Stephenson et al., 2009)
	qnrB-R	ACGATGCCTGGTAGTTGTCC		
qnrS	qnrS-F	ACGACATTCGTCAACTGCAA	417	(Stephenson et al., 2009)
	qnrS-R	TAAATTGGCACCCTGTAGGC		
qepA	qepA-F	GCAGGTCCAGCAGCGGGTAG	199	(Yamane et al., 2007)
	qepA-R	CTTCCTGCCCGAGTATCGTG		
aac(6')-Ib-cr	aac(6')-Ib-cr-F	TTGCGATGCTCTATGAGTGGCTA	482	(Park et al., 2006)
	aac(6')-Ib-cr-R	CTCGAATGCCTGGCGTGTTT		
PBRT				
IncHI1	HI1-F	GGAGCGATGGATTACTTCAGTAC	471	(Carattoli et al., 2005)
	HI1-R	TGCCGTTTCACCTCGTGAGTA		
IncHI2	HI2-F	TTTCTCCTGAGTCACCTGTTAACAC	644	(Carattoli et al., 2005)
	HI2-R	GGCTCACTACCGTTGTCATCCT		
IncI1	I1-F	CGAAAGCCGGACGGCAGAA	139	(Carattoli et al., 2005)
	I1-R	TCGTCGTTCCGCCAAGTTCGT		

Table 3. (continue)

Target	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
IncX	X-F	AACCTTAGAGGCTATTTAAG TTGCTGAT	376	(Carattoli et al., 2005)
	X-R	TGAGAGTCAATTTTTATCTCA TGTTTTAGC		
IncL/M	L/M-F	GGATGAAAACTATCAGCATCTGAAG	785	(Carattoli et al., 2005)
	L/M-R	CTGCAGGGGCGATTCTTTAGG		
IncN	N-F	GTCTAACGAGCTTACCGAAG	559	(Carattoli et al., 2005)
	N-R	GTTTCAACTCTGCCAAGTTC		
IncFIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	(Carattoli et al., 2005)
	FIA-R	GTATATCCTTACTGGCTTCCGCAG		
IncFIB	FIB-F	GGAGTTCTGACACACGATTTTCTG	702	(Carattoli et al., 2005)
	FIB-R	CTCCCGTCGCTTCAGGGCATT		
IncW	W-F	CCTAAGAACAACAAAGCCCCCG	242	(Carattoli et al., 2005)
	W-R	GGTGCGCGGCATAGAACCGT		
IncY	Y-F	AATTCAAACAACACTGTGCAGCCTG	765	(Carattoli et al., 2005)
	Y-R	GCGAGAATGGACGATTACAAAACTTT		
IncP	P-F	CTATGGCCCTGCAAACGCGCCAGAAA	634	(Carattoli et al., 2005)
	P-R	TCACGCGCCAGGGCGCAGCC		
IncFIC	FIC-F	GTGAACTGGCAGATGAGGAAGG	262	(Carattoli et al., 2005)
	FIC-R	TTCTCCTCGTCGCCAAACTAGAT		
IncA/C	A/C-F	GAGAACCAAAGACAAAGACCTGGA	465	(Carattoli et al., 2005)
	A/C-R	ACGACAAACCTGAATTGCCTCCTT		
IncT	T-F	TTGGCCTGTTTGTGCCTAAACCAT	750	(Carattoli et al., 2005)
	T-R	CGTTGATTACACTTAGCTTTGGAC		
IncFIIA	FIIs-F	CTGTCGTAAGCTGATGGC	270	(Carattoli et al., 2005)
	FIIs-R	CTCTGCCACAAACTTCAGC		
IncF	F-F	TGATCGTTTAAGGAATTTTG	270	(Carattoli et al., 2005)
	F-R	GAAGATCAGTCACACCATCC		
IncK	K-F	GCGGTCCGGAAAGCCAGAAAAC	160	(Carattoli et al., 2005)
	K-R	TCTTTCACGAGCCCGCCAAA		
IncB/O	B/O-F	GCGGTCCGGAAAGCCAGAAAAC	159	(Carattoli et al., 2005)
	B/O-R	TCTGCGTTCCGCCAAGTTCGA		

Table 3. (continue)

Target	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
IncF pMLST				
FIIs	FIIS-F	CTAAAGAATTTTGATGGCTGGC	260	(Villa et al., 2010)
	FIIS-R	CAGTCACTTCTGCCTGCAC		
FIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	(Villa et al., 2010)
	FIA-R	GTATATCCTTACTGGCTTCCGCAG		
FIB	FIBs-F	TGCTTTTATTCTTAAACTATCCAC	683	(Villa et al., 2010)
	FIB-R	CTCCCGTCGCTTCAGGGCATT		
IncI1 pMLST				
repI	repI-F	CGAAAGCCGGACGGCAGAA	142	(García-Fernández et al., 2008)
	repI-R	TCGTCGTTCCGCCAAGTTCGT		
ardA	ardA-F	ATGTCTGTTGTTGCACCTGC	501	(García-Fernández et al., 2008)
	ardA-R	TCACCGACGGAACACATGACC		
trbA	trbA-F	CGACAAATGCTTCCGGGGT	883	(García-Fernández et al., 2008)
	trbA-R	CGAATCCCTCACCATCCAG		
sogS	sogS-F	TTCCGGGGCGTAGACAATACT	291	(García-Fernández et al., 2008)
	sogS-R	AACAGTGATATGCCGTCGC		
pilL	pilL-F	CCATATGACCATCCAGTGCG	316	(García-Fernández et al., 2008)
	pilL-R	AACCACTATCTCGCCAGCAG		
IncHI1 pMLST				
HCMI1.043	P43-F	CTGGATTCCCCAGAAAAACA	570	(Phan et al., 2009)
	P43-R	TGAATCACTGCCCGTATCAA		
HCMI1.064	P64-F	ATGTGACCAACACGGAGACA	728	(Phan et al., 2009)
	P64-R	CATCGCCTTCCTGATGATCT		
HCMI1.099	P99-F	AGAAAAACGGGGACCTCAGT	590	(Phan et al., 2009)
	P99-R	GGATTGCTCACGGGAGATTA		
HCMI1.116	P116-F	TCTTCACCACGCCATATTCA	772	(Phan et al., 2009)
	P116-R	GTATCGTCATGCGGGTCTTT		
HCMI178ac	P178-F	ACTGAGCTGTTCGCGATTTT	578	(Phan et al., 2009)
	P178-R	GCGGGGTGGTTAATGTCTTT		

Table 3. (continue)

Target	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
HCMI1.259	P259-F	GAACGTAATTCCAGCGGAGA	599	(Phan et al., 2009)
	P259-R	CGCATTGTTTATGGCTACGA		
IncA/C pMLST				
repA	repA-F	AAGAGAACCAAAGACAAAGAC	982	(Hancock et al., 2017)
	repA-R	GCTGCTTACGCTTGTTGGA		
parA	parA-F	AAAAGTAATCAGCTTCGCCA	780	(Hancock et al., 2017)
	parA-R	TAGCCCACCTTCTCTAATAG		
parB	parB-F	TGTCCGAACTTGCTAAAGC	1128	(Hancock et al., 2017)
	parB-R	CTGACACAGGCACATGAA		
053	053-F	AGATCTCACAGGACATGAA	250	(Hancock et al., 2017)
	053-R	TTCAAGAACGAAGACCTGT		

Abbreviations: ESBLs, extended-spectrum beta-lactamases; PBRT, polymerase chain reaction-based replicon typing; PMCR, plasmid-mediated colistin resistance; pMLST, Plasmid multilocus sequence typing; PMQR, plasmid-mediated quinolone resistance; QRDR, quinolone resistance-determining regions.

For nucleotide sequencing analysis, PCR amplicons were purified using Nucleospin Gel® and PCR cleanup (Mccherey-Nagel) and submitted to First Base Laboratories (Selangor Darul Ehsan, Malaysia), for sequencing. DNA sequences obtained were analyzed using Chromas and Seqman programs and compared with the GenBank database using the BLAST algorithm available on the National Center for Biotechnology Information (NCBI) website.

Table 5 : PCR conditions used for genetic characterization of *Salmonella* isolates in this study

PCR target	Reaction condition					
	Initial denaturation	Denaturation	Annealling	Extension	Final extension	No. of cycles
ESBLs[a]	94°C 3 min	94°C 1 min	50°C 1 min	72°C 1 min	72°C 5 min	30
PMCR	94°C 3 min	94°C 30 sec	58°C 90 sec	72°C 1 min	72°C 10 min	25
QRDR	94°C 5 min	94°C 45 sec	57°C 45 sec	72°C 1 min	72°C 5 min	30
PMQR[b]	95°C 5 min	95°C 45 sec	55°C 45 sec	72°C 1 min	72°C 5 min	30
PBRT[c]	94°C 5 min	94°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30
IncF pMLST	94°C 5 min	94°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30
IncA/C pMLST	95°C 3 min	95°C 1 min	60°C 45 sec	72°C 1 min	72°C 5 min	30
IncHI1 pMLST	95°C 5 min	95°C 1 min	55°C 45 sec	72°C 1 min	72°C 5 min	30
IncI1 pMLST	94°C 5 min	95°C 1 min	60°C 30 sec	72°C 1 min	72°C 5 min	30

[a] bla_{CTX-M} gene was performed under ESBLs PCR condition except the annealing temperature was 55°C.

[b] *qnr* gene was performed under PMQR PCR condition except the annealing temperature was 57°C.

[c] Simplex-F was performed under PBRT PCR condition except the annealing temperature was 52°C.

1. Detection of ESBL-encoding genes

The presence of β-lactamases encoding including bla_{TEM}, $bla_{CTX-M,}$ and bla_{SHV} was investigated by PCR in ESBL positive isolate using specific primers, including bla_{TEM} (blaTEM-F/blaTEM-R), bla_{CTX-M} (blaCTX-M-F/blaCTX-M-R), and bla_{SHV} (blaSHV-F/blaSHV-R) (Chuanchuen and Padungtod, 2009). The PCR product was purified and submitted for DNA sequencing and analyzed using Blast search tool.

The thermal condition for PCR amplification ESBL-encoding genes started with the initial denaturation at 94°C for 3 minutes followed by 30 PCR cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute and extension at 72°C for 1 minute. The step for final extension was performed at 72°C for 5 minutes. The PCR amplification of bla_{CTX-M} was performed under the above PCR condition except the annealing temperature was 55°C.

2. Detection of plasmid-mediated colistin resistance genes (n=178)

All *Salmonella* strains were tested for the presence of colistin resistance encoding genes, including *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 using multiplex-PCR with specific primers, namely *mcr-1* (mcr-1F/mcr-1R), *mcr-2* (mcr-2F/mcr-2R), *mcr-3* (mcr-3F/mcr-3R), *mcr-4* (mcr-4F/mcr-4R), and *mcr-5* (mcr-5F/mcr-5R) as described previously (Rebelo et al., 2018).

The thermal condition for PCR amplification of all *mcr* genes started with the initial denaturation at 94ºC for 3 minutes followed by 25 PCR cycles of denaturation at 94ºC for 30 seconds, annealing at 58ºC for 90 seconds and extension at 72ºC for 1 minute. The step for final extension was performed at 72ºC for 10 minutes.

3. Detection of mutation in *gyrA* and *parC*

The *Salmonella* isolates resistant to ciprofloxacin and/or levofloxacin were examined for mutations in QRDR of *gyrA* and *parC* by using DNA sequencing (Chuanchuen and Padungtod, 2009). The nucleotide sequences were analyzed and compared with published *gyrA* and *parC* sequences using the BLAST search available at the NCBI website. The sequence of *gyrA* and *parC* from two ciprofloxacin and/or levofloxacin susceptible isolates were included as control.

The thermal condition for PCR amplification *gyrA* and *parC* genes started with the initial denaturation at 94ºC for 5 minutes followed by 30 PCR cycles of denaturation at 94ºC for 45 seconds, annealing at 57ºC for 45 seconds and extension at 72ºC for 1 minute. The step for final extension was performed at 72ºC for 5 minutes.

4. Detection of PMQR genes (n=178)

All the *Salmonella* isolates were examined for the presence of plasmid mediated quinolone resistance (PMQR) genes including *qnr* (*qnrA, qnrB* and *qnrS*), *aac(6')-Ib-cr* and *qepA* genes. Simplex PCR assay with specific primers (i.e., *qnrA*, qnrA-F/qnrA-R; *qnrB*, qnrB-F/qnrB-R; *qnrS*, qnrS-F/qnrS-R; *aac(6')-Ib-cr*, aac(6')-Ib-F/aac(6')-Ib-R; and *qepA,* qepA-F/qepA-R) was conducted as described previously (Park et al., 2006; Yamane et al., 2007; Stephenson et al., 2009)

The thermal condition for PCR amplification of PMQR-encoding genes started with the initial denaturation at 95ºC for 5 minutes followed by 30 PCR cycles of denaturation at 95ºC for 45 seconds, annealing at 55ºC for 45 seconds and extension at 72ºC for 1 minute. The step for final extension was performed at 72ºC for 5 minutes. The PCR amplification of *qnr* genes was performed under the above PCR condition except the annealing temperature was 57ºC.

5. Plasmid characterization

5.1 Plasmids replicon typing (PBRT) (n=178)

The PCR based replicon typing (PBRT) scheme was used to identify plasmid incompatibility (Inc) groups in all *Salmonella* isolates. Eighteen major plasmid Inc groups were screened using 5 multiplex PCRs (HI1/HI2/I1-Iγ, X/L-M/N, FIA/FIB/W, Y/P/FIC, and A-C/T/FIIA) and 3 simplex PCRs (F, K, and B/O) with specific primers (Table 2) (Carattoli et al., 2005).

The thermal condition for PCR based replicon typing started with the initial denaturation at 94ºC for 5 minutes followed by 30 PCR cycles of denaturation at 94ºC for 1 minute, annealing at 60ºC for 30 seconds and extension at 72ºC for 1 minute. The step for final extension was performed at 72ºC for 5 minutes. The PCR amplification of F replicon was performed under the above PCR condition except the annealing temperature was 52ºC.

5.2 Plasmid multilocus sequence typing (pMLST) (n=19)

Currently, the Plasmid MLST scheme was available for 6 Inc groups including A/C, I1, HI1, HI2, F, and N replicons. Therefore, based on the results from PBRT in 5.1, the isolates with identified replicon type of the following groups: IncA/C, IncI1, IncHI1, IncHI2, IncF and IncN plasmids and exhibited resistance to at least one antimicrobial agent were selected for sequence typing by pMLST (García-Fernández et al., 2008; Phan et al., 2009; Villa et al., 2010; Hancock et al., 2017). All PCR amplifications were performed using the primers listed in Table 2. Analysis of pMLST of each plasmid was carried out by matching multiple target genes on plasmid DNA sequences with the Plasmid MLST schemes available at http://pubmlst.org/plasmid/. In this study the pMLST was performed in 4 Inc groups including IncI1 (n=5), IncHI1 (n=4), IncA/C (n=1), and IncF (n=9). An overview of pMLST process is detailed in Figure 2.

The IncI1 MLST scheme was used 5 genes for sequence typing including *repI, ardA, trbA, sogS,* and *pilL*. PCR amplification of these target genes for IncI1 MLST were performed as follows: Initial denaturation at 94ºC for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72ºC for 1 minute and final extension at 72ºC for 5 min.

The IncHI1 MLST scheme was covered 6 loci including HCMI1.043, HCMI1.064, HCMI1.099, HCMI1.116, HCMI1.178ac, and HCMI1.259. The thermal condition for PCR amplification of 6 target loci for IncHI1 MLST included initial denaturation at 95ºC for 5 minutes followed by 30 PCR cycles of denaturation at 95ºC for 45 seconds, annealing at 55ºC for 45 seconds and extension at 72ºC for 1 minute. The step for final extension was performed at 72ºC for 5 minutes.

The IncA/C MLST scheme used 4 genes including *repA, parA, parB,* and *053*. PCR amplifications of the target genes for IncA/C MLST were performed as follows: Initial denaturation at 95ºC for 3 minutes, followed by 30 cycles of denaturation at 95ºC for 1 minute, annealing at 60ºC for 45 seconds, extension at 72ºC for 1 minute and the final extension at 72ºC for 5 min.

The IncF replicon sequence typing (RST) was unique and separately performed due to multi-replicon nature of these plasmids. The isolates carrying FII, FIIs, FIA, FIB were included. The sequence variants identified for each replicon (FII, FIIs, FIA and FIB) were assigned to an allele number. Each plasmid can be defined as the FAB (FII, FIA, FIB) formula determined by the allele type and number identified for each replicon. For instance, the formula S1:A1:B:1 was assigned from FIIs allele 1, FIA allele 1, and FIB allele 1. The formula S1:A-:B- was assigned from FIIs allele 1. The A- and B- symbols mean the absence of FIA and FIB replicons, respectively.

All PCR amplifications for IncF replicon sequence typing were performed with following condition: Initial denaturation at 94ºC for 5 minutes, followed by 30 cycles of denaturation at 94ºC for 1 minute, annealing at 60ºC for 30 seconds, elongation at 72ºC for 1 minute and the final step 1 cycle at 72ºC for 5 minutes.

Fasta files from individual allele-specific sequencing reads were submitted for identification of pMLST allele number and ST by comparison with pMLST database (http://pubmlst.org/plasmid/). Then, the plasmid sequence types (ST) of each plasmid were determined based on the combination of alleles identified. An example of pMLST data interpretation (IncA/C) is detailed in Figure 3.

Identification of IncF replicon sequence type used FAB (FII, FIA, FIB) formula determine by the combination of allele type identified in each replicon. Example of IncF RST data interpretation was detailed in Figure 4.

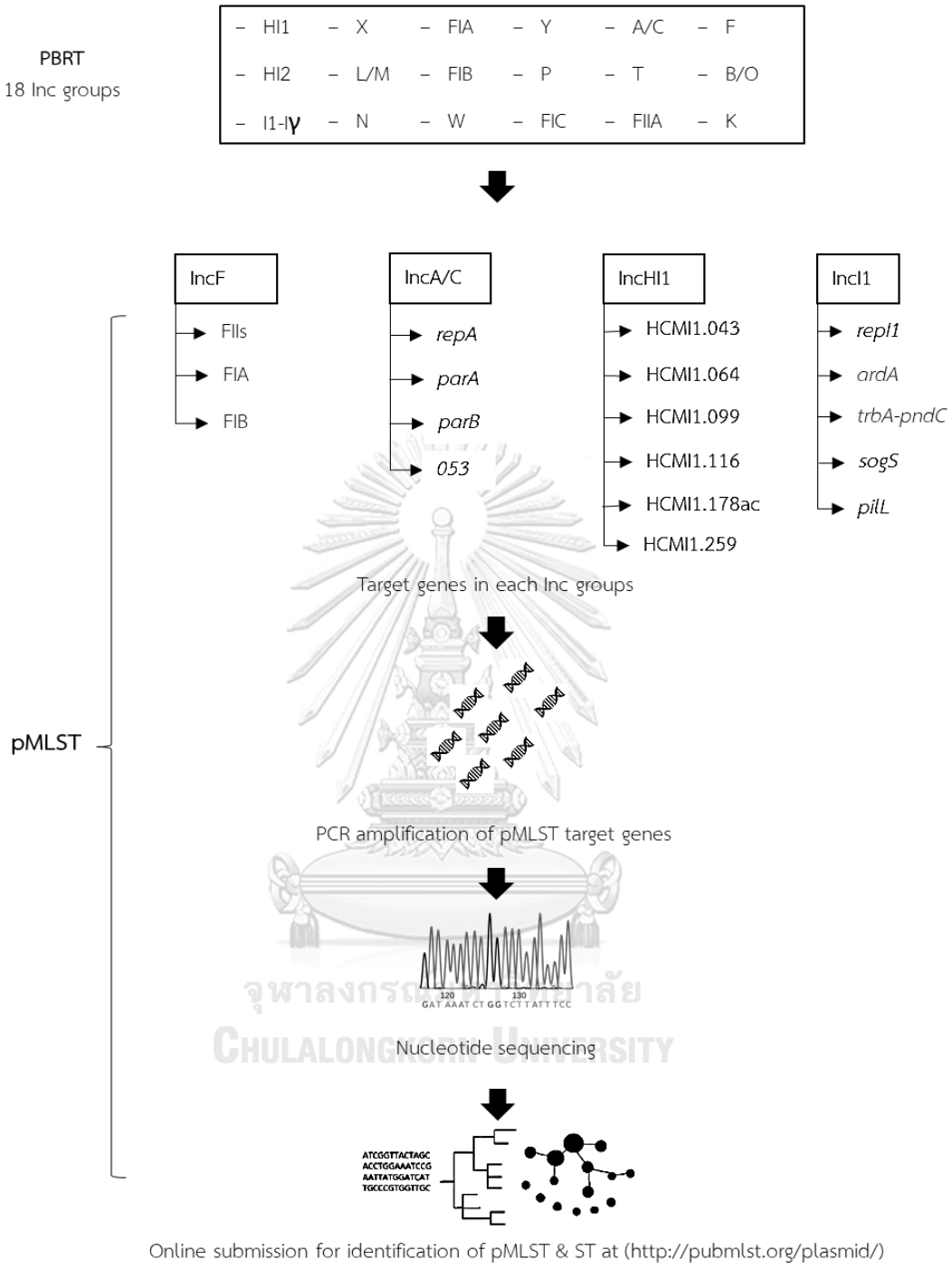
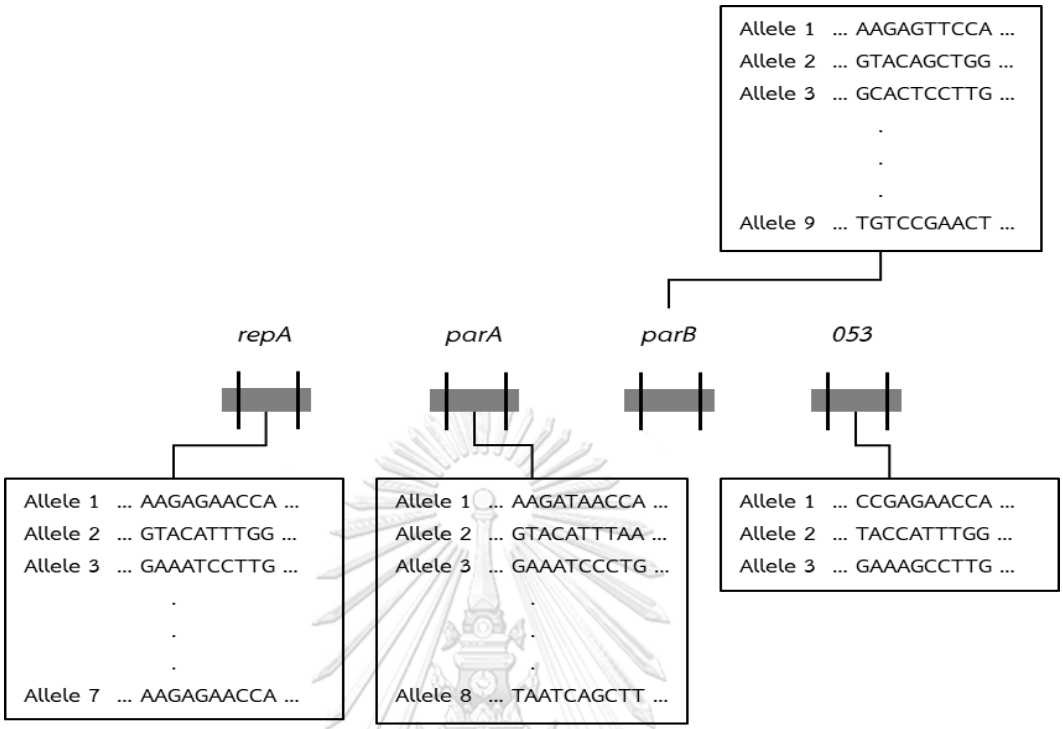


Figure 2: PBRT and pMLST scheme. Inc groups identification by PBRT was first conducted, followed by PCR amplification and nucleotide sequencing of pMLST target genes for each Inc groups. Nucleotide sequences were submitted to pMLST online database for ST identification.

(A)

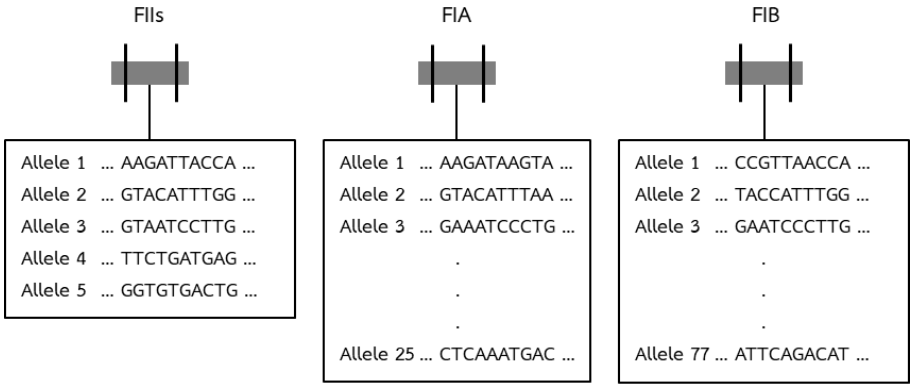


(B)

Isolate No	Sequence Types (ST)	Allelic type of individual target genes of IncA/C plasmid			
		repA	parA	parB	053
1	3	2	2	2	1
2	4	2	2	3	1
3	10	4	5	6	2
4	13	7	8	9	3

Figure 3 : pMLST of IncA/C. (A) Sequences from four target genes of IncA/C plasmid including *repA*, *parA*, *parB*, and *053* were used. Allele numbers are assigned to unique sequences of individual genes. The allele number combination was used to define a sequence type (ST). (B) Each sequence type is a unique combination of allele number. More than 10 IncA/C-ST data are available, Examples are given for ST3, ST4, ST10, and ST13.

(A)



(B)

Isolate No	Allele numbers for replicons			FAB Formula[a]
	FIIs	FIA	FIB	
1	2	1	4	S2:A1:B4
2	1	-	47	S1:A-:B47
3	3	-	-	S3:A-:B-

[a]Allele in each replicon (FIIs, FIA, and FIB) were identified and numbered. FAB formula was assigned by combinations of allele number observed among the replicons.

-, The A- and/or B- symbols mean the absence of FIA and FIB replicons.

Figure 4 : pMLST of IncF. (A) Sequences from three replicons of IncF plasmid (including FIIs, FIA, and FIB) were characterized by Replicon Sequence Typing (RST). All three replicons were sequenced, and unique sequences of individual replicons were used to assign allele number. (B) The combination of allele numbers of each replicon was used to define FAB (FIIs, FIA, FIB) formula. The examples are given for S2:A1:B4, S1:A-:B47, and S3:A-:B- formula.

6. Statistical analysis

The percentage of AMR rates and prevalence of replicon types in this study were analyzed by descriptive analysis. Association between *Salmonella* carrying plasmids and AMR phenotypes were determined using Chi-square test by SPSS program version 22.0. A *p*-value <0.05 was considered statistically significant.

CHAPTER IV RESULTS

1. *Salmonella* serotypes

Of all the *Salmonella* isolates tested (n=178), 77 serovars were identified. Serovar Weltevreden was predominant (9.6%), followed by serovar Stockholm (9.0%), Typhimurium (7.3%), Rissen (5.6%), and Stanley (4.5%) (Table 5). Serovar Weltevreden was most common among the isolates from dogs (n=16). Serovars Choleraesuis (n=1) was isolated from a dog. Enteritidis (n=5) was isolated from 5 dogs. Serovars Typhimurium (n=13) and Weltevreden (n=17) were isolated from both dogs (n=16) and cats (n=1). These two serovars together with Rissen, Stanley, and Stockholm were found in both isolates from dogs and cats.

Table 6 : *Salmonella* serovars isolated from dogs and cats in Northern and Northeastern Thailand (n=178)

Salmonella	**Northern (n=36)**		**Northeastern (n=142)**		**Total**
serovars	**Dogs (%)**	**Cats (%)**	**Dogs (%)**	**Cats (%)**	**number (%)**
Aberdeen			1(0.7)		1(0.6)
Agona	2(5.5)		4(2.8)		6(3.4)
Amberstra				1(0.7)	1(0.6)
Aminatu			3(2.1)		3(1.7)
Amounderbess			1(0.7)		1(0.6)
Amsterdam			2(1.4)		2(1.1)
Anatum			3(2.1)		3(1.7)
Athinai			1(0.7)		1(0.6)
Bardo			3(2.1)		3(1.7)
Biafra			2(1.4)		2(1.1)
Blegdam			2(1.4)		2(1.1)
Braenderup			1(0.7)		1(0.6)
Brilla	1(2.8)			1(0.7)	2(1.1)
Bury			1(0.7)		1(0.6)
Chincol			1(0.7)		1(0.6)
Choleraesuis	1(2.8)				1(0.6)

Table 5. (continued)

Salmonella	Northern (n=36)		Northeastern (n=142)		Total
serovars	Dogs (%)	Cats (%)	Dogs (%)	Cats (%)	number (%)
Clanvillian			1(0.7)		1(0.6)
Dallgow			1(0.7)		1(0.6)
Damman			1(0.7)		1(0.6)
Derby			1(0.7)		1(0.6)
Dublin			1(0.7)		1(0.6)
Eastbourne	1(2.8)		3(2.1)		4(2.2)
Eingedi	1(2.8)				1(0.6)
Enteritidis	3(8.3)		2(1.4)		5(2.8)
Farsta			1(0.7)		1(0.6)
Frankfurt			1(0.7)		1(0.6)
Galil			1(0.7)		1(0.6)
Give			1(0.7)		1(0.6)
Hadar			1(0.7)		1(0.6)
Hill			1(0.7)		1(0.6)
Hissar			2(1.4)		2(1.1)
Hvittingdoss			1(0.7)		1(0.6)
Kedougou			2(1.4)		2(1.1)
Kingston			1(0.7)		1(0.6)
Kisangami			1(0.7)		1(0.6)
Kisii			1(0.7)		1(0.6)
Lekke	1(2.8)				1(0.6)
Lomita	1(2.8)				1(0.6)
Madras	1(2.8)				1(0.6)
Malaka			1(0.7)		1(0.6)
Megumeri			1(0.7)		1(0.6)
Menden	2(5.6)				2(1.1)
Moers			1(0.7)		1(0.6)
Mokola			1(0.7)		1(0.6)
Montevideo			1(0.7)		1(0.6)
Nyborg			1(0.7)		1(0.6)
Onarimon			1(0.7)		1(0.6)

Table 5. (continued)

Salmonella	Northern (n=36)		Northeastern (n=142)		Total
serovars	Dog (%)	Cats (%)	Dogs (%)	Cats (%)	number (%)
Onireke			2(1.4)		2(1.1)
Oslo			1(0.7)		1(0.6)
Othmarschen	1(2.8)		3(2.1)		4(2.2)
Parathyphi B	1(2.8)	1(2.8)	2(1.4)	1(0.7)	5(2.8)
Regent			1(0.7)		1(0.6)
Ridge			1(0.7)		1(0.6)
Rissen			8(5.6)	2(1.4)	10(5.6)
Ruzizi			1(0.7)		1(0.6)
Saloniki			1(0.7)		1(0.6)
Saphra			1(0.7)		1(0.6)
Seremban	2(5.6)				2(1.1)
Stanley	1(2.8)		5(3.5)	1(0.7)	7(3.9)
Stockholm	6(16.7)	3(8.3)	6(4.2)	1(0.7)	16(9.0)
Stuttgart				1(0.7)	1(0.6)
Tees			1(0.7)		1(0.6)
Tilburg	1(2.8)		2(1.4)		3(1.7)
Tokoin			1(0.7)		1(0.6)
Truvo			1(0.7)		1(0.6)
Typhimurium			12(8.4)	1(0.7)	13(7.3)
Uppsala	2(5.6)		1(0.7)		3(1.7)
Utah			1(0.7)		1(0.6)
Vilvoorde			1(0.7)		1(0.6)
Virchow		1(2.8)	1(0.7)		2(1.2)
Weltevreden	1(2.8)		15(10.6)	1(0.7)	17(9.6)
Westafrica			1(0.7)		1(0.6)
Weston		1(2.8)			1(0.6)
Wimborne			1(0.7)		1(0.6)
Winston		1(2.8)			1(0.6)
Worthington			1(0.7)		1(0.6)
Yeerongpilly			2(1.4)	2(1.4)	4(2.2)
Total	**29**	**7**	**130**	**12**	**178**

2. Antimicrobial resistance phenotype

2.1 Antimicrobial susceptibilities among *Salmonella* from dogs and cats (n=178)

Of all the *Salmonella* isolates tested, 86 isolates (48.3%) were resistant to at least one antimicrobial agent and 61 isolates (34.3%) were MDR (Figure 5). The majority of the isolates exhibited resistance to ampicillin (38.8%), followed by tetracycline (37.1%), sulfamethoxazole (34.3%), streptomycin (30.3%), and trimethoprim (20.2%). Resistance to colistin was limited (1.1%). Resistance to cephalosporins (ceftazidime, 1.1%; cefpodoxime, 1.1%; ceftriaxone, 0.6% and cefotaxime, 0.6%) was found at low rates. All the isolates were susceptible to ciprofloxacin, levofloxacin, meropenem and imipenem. Two dog isolates (i.e., a serovar Weltevreden and a serovar Regent) were resistant to ceftazidime (1.1%). A serovar Stockholm from dog and a serovar Rissen from cat were resistant to cefpodoxime. Three cat isolates (1.7%) and 58 dog isolates (32.6%) were MDR. A total of 31 AMR patterns of *Salmonella* isolates from dogs and cats were observed in this study (Table 6). The most common AMR patterns were observed only in dog isolates including AMP-STR-SUL-TET-TRI (6.7%), followed by AMP-STR-SUL-TET (6.2%) and AMP-CHP-STR-SUL-TET-TRI (4.5%).

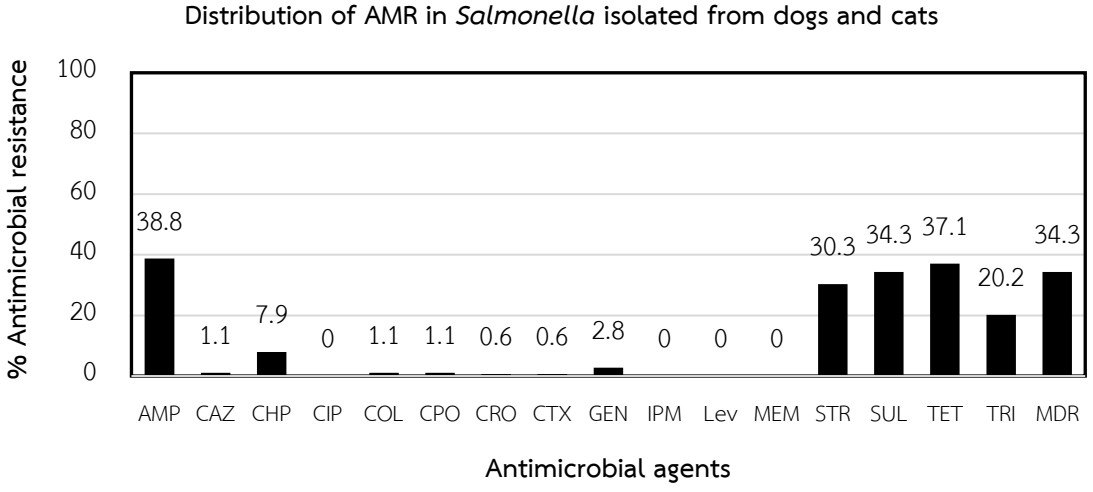


Figure 5 : Frequency of antimicrobial resistance in *Salmonella* isolated from dogs and cats (n=178). Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CHP, chloramphenicol; CIP, ciprofloxacin; COL, colistin; CPO, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; GEN, gentamicin; IPM, Imipenem; Lev, Levofloxacin; MEM, Meropenem; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; TRI, trimethoprim; MDR, multidrug resistance.

Table 7 : Antimicrobial resistance pattern of *Salmonella* isolated from dogs and cats (n=178)

Resistance pattern	No. of isolates (%)	
	Dogs (n=159)	Cats (n=19)
AMP	3 (1.7)	0
GEN	1 (0.6)	0
STR	4 (2.2)	0
SUL	1 (0.6)	0
TET	1 (0.6)	0
AMP-STR	1 (0.6)	1 (0.6)
AMP-SUL	2 (1.1)	1 (0.6)
AMP-TET	3 (1.7)	0
AMP-TRI	1 (0.6)	0
CAZ-TET	1 (0.6)	0
STR-TET	2 (1.1)	0
SUL-TET	3 (1.7)	0
AMP-STR-SUL	1 (0.6)	1 (0.6)
AMP-STR-TET	4 (2.2)	0
AMP-STR-TRI	1 (0.6)	0
AMP-SUL-TET	2 (1.1)	0
STR-SUL-TET	2 (1.1)	0
AMP-CHP-SUL-TRI	1 (0.6)	0
AMP-COL-SUL-TET	1 (0.6)	0
AMP-STR-SUL-TET	11 (6.2)	0
AMP-STR-SUL-TRI	1 (0.6)	0
AMP-STR-TET-TRI	1 (0.6)	-
AMP-SUL-TET-TRI	6 (3.4)	2 (1.1)
AMP-CHP-GEN-SUL-TET	2 (1.1)	0
AMP-CPD-SUL-TET-TRI	0	1 (0.6)
AMP-STR-SUL-TET-TRI	12 (6.7)	0
CAZ-CHP-STR-SUL-TET	1 (0.6)	0
AMP-CHP-STR-SUL-TET-TRI	8 (4.5)	0
AMP-COL-STR-SUL-TET-TRI	1 (0.6)	0
CHP-GEN-STR-SUL-TET-TRI	1 (0.6)	0
AMP-CHP-CTX-CPD-CRO -GEN-STR-TET	1 (0.6)	0
Total	**178**	

Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CHP, chloramphenicol; CIP, ciprofloxacin; COL, colistin; CPO, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; GEN, gentamicin; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; TRI, trimethoprim

2.2 ESBL – producing *Salmonella* from dogs and cats

Only the serovar Stockholm from a dog isolate in this study was resistant to cefotaxime and cefpodoxime and confirmed to be ESBL-producer.

3. Genes underlying ESBL production and AMR

3.1 Detection of ESBL-encoding genes

The ESBL-positive serovar Stockholm from dog was found to harbor $bla_{\text{CTX-M55}}$ of CTX-M group 1. The $bla_{\text{TEM-1}}$ gene encoding broad spectrum β-lactamase was additionally detected in this ESBL-producer.

3.2 Plasmid-mediated colistin resistance genes (n=178)

Of all *Salmonella* isolates tested, none of the isolates were positive in all *mcr* genes tested (i.e., *mcr-1, mcr-2, mcr-3, mcr-4,* and *mcr-5*).

3.3 PMQR genes

Of the PMQR genes tested, only *qnrS* was detected in 18 *Salmonella* isolates from dogs (18/178, 10.1%) (ciprofloxacin MIC range = 0.015625–1 μg/mL and levofloxacin MIC range = 0.0625-1 μg/mL). Mutations in QRDRs were not performed in this study due to all isolates were susceptible to quinolone drugs tested (i.e., ciprofloxacin and levofloxacin).

4. Characteristics of plasmids identified in *Salmonella* from dogs and cats

4.1 Plasmid replicons among the *Salmonella* isolates from dogs and cats (n=178)

Based on the replicon types detection in all *Salmonella*, 5 out of 18 plasmid replicon types including IncA/C. IncFIIA, IncHI1, IncI1, and IncN were detected in 60 isolates (33.7%). IncFIIA was most prevalent (51 isolates, 28.7%), IncI1-Iγ (6 isolates, 3.4%), IncHI1 (4 isolates, 2.2%), IncN (2 isolates, 1.1%) and IncA/C (1 isolate, 0.6%) were detected at low level (Figure 6). Among these 60 replicon identifiable isolates, 4 out of 60 (6.7%) contained more than one replicon type. Up to 2 replicon types were found in one isolate. IncFIIA was most common replicon type in the isolates from dogs and cats, the IncHI1 was most commonly detected in the *Salmonella* isolates carrying *qnrS* (4 isolates, 2.2%). Among the AMR isolate (n=86), 16 isolates (18.6%) were contained at least one plasmid in Inc groups identified (Table 7). A chi-square test was performed to examine the relationship between *Salmonella* carrying plasmids and resistance phenotypes. The relationship between these circumstances was significant, ($p< 0.05$). *Salmonella* isolates that carrying plasmid were more likely to be resistant.

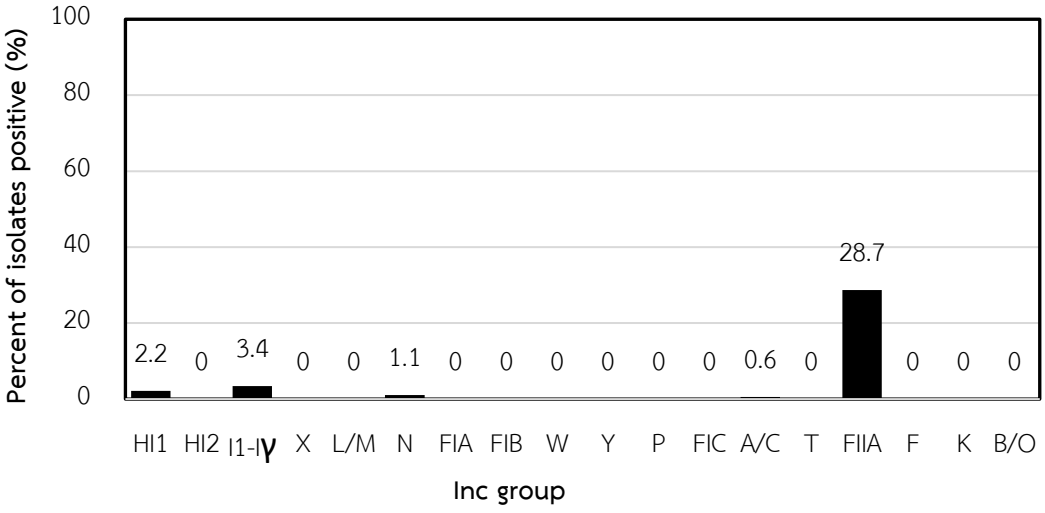


Figure 6 : Distribution of plasmid replicon type of *Salmonella* Isolates from dogs and cats (n=178)

Table 8 : Plasmid replicon types and resistance genes detected in AMR *Salmonella* isolates (n=16)

ID	Source	Inc/rep	Serotype	Resistance pattern	Resistance genes		
					PMQR	β-lactamase	*mcr*
29	dog	A/C	Regent	CAZ-CHP-STR-SUL-TET	*qnrS*	-	-
21		FIIAs	Weltevreden	TET	-	-	-
26		FIIAs	Eastbourne	SUL-TET	-	-	-
42		FIIAs	Othmarschen	AMP-SUL-TET-TRI	-	-	-
88		FIIAs	Weltevreden	STR-SUL-TET	-	-	-
109		FIIAs	Bardo	STR	-	-	-
138		FIIAs	Seremban	AMP	-	-	-
170		FIIAs	Stockholm	SUL-TET	-	-	-
100		HI1	Biafra	AMP-CHP-STR-SUL-TET-TRI	*qnrS*	-	-
101		HI1	Anatum	AMP-CHP-STR-SUL-TET-TRI	*qnrS*	-	-
102		HI1	Anatum	AMP-CHP-STR-SUL-TET-TRI	*qnrS*	-	-
103		HI1, I1-Iγ	Anatum	AMP-CHP-STR-SUL-TET-TRI	*qnrS*	-	-
63		I1-Iγ	Malaka	AMP-TET	-	-	-
47		I1-Iγ, FIIAs	Blegdam	AMP-TET	*qnrS*	-	-
85	Cat	I1-Iγ	Typhimurium	AMP-STR-SUL	-	-	-
5		I1-Iγ, FIIAs	Weltevreden	AMP-SUL	-	-	-

Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CHP, chloramphenicol; STR, streptomycin; SUL,sulfamethoxazole; TET, tetracycline; TRI, trimethoprim

-, not detected

4.2 Plasmid multilocus sequence type (pMLST)

4.2.1 Replicon sequence type (RSTs) of IncF plasmids in the *Salmonella* isolates (n=9)

The RST scheme was previously developed for subtyping F-type plasmids due to their multiple replicon status. Each plasmid was identified using the FAB (FIIs, FIA, FIB) formula. The pMLST analysis revealed that 9 AMR *Salonella* isolates that belong to F replicon contained various IncF-replicon sequence types. Four different combinations of replicons were identified, which included S1:A-:B- (n=4), S1:A-:B22 (n=2), S3:A-:B- (n=1), and S-:A-B:47 (n=1). Only 2 FIIs replicons (i.e., ID 26 and 42) were non-typable by pMLST due to no matching allelic number from pMLST database but also showed closest match to FIIs:4. The *qnrS* gene was present among these 9 isolates, 1 of these plasmids carrying *qnrS* was found in *Salmonella* Blegdam from a dog (Table 8).

Table 9 : Characteristics of AMR *Salmonella* carrying IncF plasmid replicon alleles (FIIs, FIA, and FIB) (n=9)

Source	ID	Serovars	Allele numbers for replicons[a]			FAB	Resistance	Resistance
			FIIs	FIA	FIB	Formula	gene	phenotype
dog	21	Weltevreden	1	neg	neg	S1:A-:B-	-	TET
	26	Eastbourne	NT	neg	47	S-:A-:B47	-	SUL-TET
	42	Othmarschen	NT	neg	neg	-	-	AMP-SUL-TET-TRI
	47	Blegdam	1	neg	22	S1:A-:B22	*qnrS*	AMP-TET
	88	Weltevreden	1	neg	neg	S1:A-:B-	-	STR-SUL-TET
	109	Bardo	3	neg	neg	S3:A-:B-	-	STR
	138	Seremban	1	neg	22	S1:A-:B22	-	AMP
	170	Stockholm	1	neg	neg	S1:A-:B-	-	SUL
cat	5	Weltevreden	1	neg	neg	S1:A-:B-		AMP-SUL

[a]Allele variants for each replicon sequence

NT, non-typeable

-, not detected

Abbreviations: AMP, ampicillin; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; TRI, trimethoprim

4.2.2 Characteristics of pMLST in the *Salmonella* isolates carrying A/C, HI1, and I1 replicons (n=10)

Characteristics of the plasmid RST are shown in Table 9. Plasmid STs were successfully determined in the *Salmonella* isolates containing IncA/C (n=1), IncHI1 (n=4), and IncI1 (n=5) plasmids. The A/C replicon was detected in serovar Regent originated from 1 dog. According to the A/C pMLST scheme, this plasmid was assigned to ST 6.

All *Salmonella* isolates carrying IncHI1 plasmid (n=4) was contained *qnrS* genes. All were originated from dog. Based on the HI1 pMLST typing scheme, the novel ST of IncHI1 plasmid was identified in this study and deposited in the pMLST database as ST16 (https://pubmlst.org/bigsdb?db=pubmlst_plasmid_isolates&page=profiles).

All *Salmonella* containing IncI1 plasmids (n=5) were obtained from dogs. The plasmids were assigned to 3 different sequence types, including ST60, ST101, and ST136. In addition, 2 incomplete IncI1 pMLST profiles were observed due to no perfect matching ST with pMLST database.

The plasmids containing *qnrS* were detected in 7 out of 10 *Salmonella* with resistant plasmid isolated from dog, including IncA/C (n=1), IncI1 (n=2), and IncHI1 (n=4). Seven *Salmonella* isolates carrying IncHI1 (n=4), A/C (n=1) and I1 (n=2) were MDR (Table 9).

Table 10 : Characteristics of the plasmid STs in *Salmonella* carrying IncA/C, IncHI1, and IncI1 plasmid from dogs (n=10)

ID	Serovars	Inc/rep	Plasmid ST[a]	Allele type	Resistance gene	Resistance phenotype
29	Regent	A/C	6	*repA*, 2; *parA*, 2; *parB*, 5; *053*, 1	*qnrS*	CAZ-CHP-STR-SUL-TET
100	Biafra	HI1	16	HCM1.043, 1; HCM1.064, 1; HCM1.099,1; HCM1.116, 1; HCM1.178ac, 0; HCM1.259, 1	*qnrS*	AMP-CHL-STR-SUL-TET-TRI
101	Anatum	HI1	16	HCM1.043, 1; HCM1.064, 1; HCM1.099,1; HCM1.116, 1; HCM1.178ac, 0; HCM1.259, 1	*qnrS*	AMP-CHL-STR-SUL-TET-TRI
102	Anatum	HI1	16	HCM1.043, 1; HCM1.064, 1; HCM1.099,1; HCM1.116, 1; HCM1.178ac, 0; HCM1.259, 1	*qnrS*	AMP-CHL-STR-SUL-TET-TRI
103	Anatum	HI1	16	HCM1.043, 1; HCM1.064, 1; HCM1.099,1; HCM1.116, 1; HCM1.178ac, 0; HCM1.259, 1	*qnrS*	AMP-CHL-STR-SUL-TET-TRI
5	Weltevreden	I1	101	*repI1*, 2; *ardA*, 1; *trbA*, 4; *sogS*, 1; *pilL*, 2	-	AMP-SUL
47	Blegdam	I1	136	*repI1*, 2; *ardA*, 2; *trbA*, 24; *sogS*, 9; *pilL*, 1	*qnrS*	AMP-SUL
83	Malaka	I1	60	*repI1*, 1; *ardA*, 2; *trbA*, 8; *sogS*, 6; *pilL*, 3	-	AMP-TET
85	Typhimurium	I1	U	*repI1*, 2; *ardA*, 7; *trbA*, 4; *sogS*, 1; *pilL*, 2	-	AMP-STR-SUL-TET
101	Anatum	I1	U	*repI1*, 1; *ardA*, 2; *trbA*, 20; *sogS*,6; *pilL*, 3	*qnrS*	AMP-CHL-STR-SUL-TET-TRI

[a]Sequence type (ST) was assigned to the different combinations of allele variants observed in the plasmids.

U, untypeable.

-, not detected.

Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CHP, chloramphenicol; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; TRI, trimethoprim

CHAPTER V DISCUSSION

One of the major findings of this study was the wide variation of *Salmonella* serovars and distribution of MDR *Salmonella* among pet dogs and cats in Northern and Northeastern Thailand. It should be noted that, due to the limited number of the *Salmonella* isolates included in this study, the results may not be able to represent the characteristics of the pathogen in dogs and cats in the whole region. However, the data is considered useful and in demand because of the limited availability of data of *Salmonella* in pet origins in the present time.

Salmonella serotyping has been recognized as important tool for monitoring endemic *Salmonella* serovars and providing useful epidemiological data on linkage to the sources of salmonellosis outbreak. In this study, up to 77 serovars were identified, of which the predominant *Salmonella* serovars were Weltevreden (9.6%), Stockholm (9.0%), and Typhimurium (7.3%). These serovars are of medical importance that are implicated in human salmonellosis in many countries (Brown et al., 2017; Li et al., 2018). In comparison to the previous report in the same region (serovars Weltevreden, 15.6%) and Typhimurium, 13.9%), the percentage of both serovars Weltevreden and Typhimurium in this study was lower (Srisanga et al., 2017). At the same time, serovars Stockholm was not observed in the previous study. The difference of the serovars detected could be associated with many factors e.g., different time of sampling, animal population, random selection of the testing isolates etc. The current study covers the isolates obtained between 2015-2018, while those in the previous study were collected during 2012-2015. This different sampling period may result in the serovars variations.

Serovar Weltevreden was the most common serovars among pet dogs and cats previously reported in Northeastern Thailand (Polpakdee et al., 2012; Srisanga et al., 2017), in agreement with this study. *Salmonella* Weltevreden has recently emerged as a dominant serovar, causing in humans salmonellosis in South-East Asia and Europe, where it has been linked with contaminated sea food (Ponce et al., 2008), aquatic foods (Hounmanou et al., 2020), vegetables and water (Thong et al.,

2002). These supported the hypothesis that the potential sources of serovar Weltevreden are aquatic production-related (Hounmanou et al., 2020).

Another serovars of public health importance including Choleraesuis, Enteritidis, and Typhimurium were observed. These serotypes are not commonly detected in companion animals. However, all three serovars were among top-ten most common serovars from Thai patients (Hendriksen et al., 2009). The *Salmonella* isolates in this study were originated from clinically healthy dogs and cats, indicating that these pet animals serve as asymptomatic reservoirs of medically-important *Salmonella* serovars. Therefore, close and frequent contact between owners and their pets potentiate *Salmonella* infection in humans and place *Salmonella* in pet carriers as a public health issue (Leonard, 2014).

In this study, serovars Choleraesuis was found only in dog (0.6%). Despite a very low rate, it represents an important veterinary public health issue. This is because serovars Choleraesuis causes Paratyphoid in pig and highly pathogenic to humans (Chiu et al., 2002). In previously reported, *S.* Choleraesuis ssp. *arizonae* has been isolated from stray dogs in the urban area of Northwest Mexico (Castro et al., 2019) suggested that stray dogs with free access to defecation in any area could promote the spread of the pathogen to other animals as well as humans.

The detection of *Salmonella* from dogs could correspond to serovars of public health importance indicating a potential risk for humans. Serovar Enteritidis is a major cause of salmonellosis worldwide. The pathogen is commonly found in poultry (Herikstad et al., 2002). Poultry has been considered the most importance source of serovars Enteritidis, in agreement with this study where serovars Enteritidis was found at limited rates (2.8%). Serovars Typhimurium has a broad host range and causes disease in both animals and humans. This serovars is common in Europe and The United States (Herikstad et al., 2002) and currently a major public health problem in developing countries (Herikstad et al., 2002). In Thailand, serovars Typhimurium accounted for 5% of salmonellosis in humans and the incidence rate does not have tendency to increase (Herikstad et al., 2002).

In comparison with same regions in difference samples, predominant serovars in previous report from Northern and Northeastern Thailand were Kentucky, Give,

and Typhimurium in broilers and Rissen, Typhimurium, and Weltevreden in pigs (Phongaran et al., 2019) and also Enteritidis and Cholerasuis from humans isolates (Whistler et al., 2018). In Southern region, the most common serovars isolated from pork, chicken meat, and fresh vegetables were Rissen, Albany, and Typhimurium, respectively (Lertworapreecha et al., 2012). Moreover, previous report in central Thailand has been reported the common serovars from humans isolates including, Weltevreden, Stanley, Anatum, Rissen, Enteritidis, Typhimurium, and Choleraesuis (Sirichote et al., 2010). Thus, the serovars of *Salmonella* from pet dogs and cats in this study is consistent with other reports, and the serovars detected herein are among the predominant serovars reported from other regions in Thailand, including serovars Weltevreden as well. This highlights that *Salmonella* serotyping is important and further investigate are necessary.

Almost half of the *Salmonella* isolates from pet dogs and cats in this study (47.78%) were resistant strains and this was lower than the previous report in dogs from Ethiopia (90.5%) (Kiflu et al., 2017). It is consistent with a report in Thailand (54.8%) (Polpakdee et al., 2012). The prevalence of *Salmonella* isolates that were resistant to multiple drugs (34.3%), of which serovar Typhimurium (16.4%) was most frequently - identified MDR isolates. High resistance rates to ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim comparison in this study were similar to those in previous studies in Thailand (Polpakdee et al., 2012; Srisanga et al., 2017; Wu et al., 2020). No ciprofloxacin and levofloxacin resistance was observed in our study, in agreement with previous studies (Polpakdee et al., 2012; Yoon et al., 2017). In contrast, streptomycin resistance rate in this study (30.3%) was much higher than previous studies in pet dogs and cats in Thailand (Wu et al., 2020) and pet dogs in Ontario (Leonard et al., 2011). Streptomycin is an antimicrobial agent of high importance in human medicine and used to treat a variety of infections.

In comparison to the AMR studies in *Salmonella* isolates from pork, chicken meat, and humans in Northeastern Thailand (Sinwat et al., 2015), resistance rates to most antimicrobials tested in this study (ciprofloxacin, chloramphenicol, and gentamicin) were lower than those pork, chicken meat, and humans. However, the similar AMR patterns (e.g., ampicillin, streptomycin, sulfamethoxazole, tetracycline,

and trimethoprim) were observed, especially, in serovar Rissen and Anatum. In addition, the previous study in Northern Thailand reported that predominant serovar isolated from pork and pig farm were Rissen, Stanley, Typhimurium, Weltevreden and these *Salmonella* isolates exhibited high resistance rate to ampicillin, tetracycline, and streptomycin too (Tadee et al., 2015; Patchanee et al., 2016). Distribution of MDR *Salmonella* serovars isolated from different sources in Northern and Northeastern were detailed in figure 7.

ESBL-producing *Salmonella* posed a serious public health concern because cephalosporins are drug of choice in treatment AMR infection and salmonellosis. In this study, only one ESBL producing *Salmonella* isolate, serovar Stockholm, was observed. As antibiotic history in pet dogs and cats sampled was not revealed, it can be probably expected that use of cephalosporins is less common in small animals in this region. The latter is likely due to their high price. The ESBL-producing serovar Stockholm carried $bla_{\text{CTX-M-55}}$ gene, the variant of $bla_{\text{CTX-M-15}}$ with high efficiency against ceftazidime (Ma et al., 2018; Nadimpalli et al., 2019) and $bla_{\text{TEM-1}}$. It was previously shown that $bla_{\text{CTX-M-55}}$ is often associated with $bla_{\text{TEM-1}}$ on MDR plasmids identified in *Salmonella* and *E. coli* from both humans and food animals in Thailand (Trongjit et al., 2017; Pungpian et al., 2020). Despite the limited occurrence, ESBL-producing *Salmonella* with ESBL gene has an important implication and can be transmitted to another host, even among low numbers of bacteria (Guardabassi et al., 2004; Liu et al., 2019).

PMQR has been shown to confer low-level resistance of quinolones (Wong et al., 2014). In the present study, only *qnrS* was detected (10.1%). It was previously described that the *qnr* genes do not always confer resistance level of fluoroquinolone based on clinical breakpoint (Ferreira et al., 2018). In spite of the low prevalence of PMQR genes observed, this is of concern as the genes can be associated with other mechanisms (e.g., target site mutations) and promote the accumulation effect for higher-level quinolone resistance (Wong et al., 2014). It has been suggested that dissemination of *qnr* genes could play a role in co-selection of MDR phenotype in bacteria as they can co-localize with other resistance genes on the same mobile genetic elements (MGE). In addition, the co-harbouring resistance

and virulence genes may result in resistant *Salmonella* serovars with more virulence (Virolle et al., 2020).

Plasmids are important vehicles for the carriage of other mobile genetic element (MGE) and acquiring antimicrobial resistance genes among bacterial population. Identification and characterization of plasmid can provide the comprehensive picture of plasmids contributed to the dissemination of AMR determinants and horizontal spread of AMR (Rozwandowicz et al., 2018). Characterization of plasmid Inc groups has been used as tool for epidemiological study to monitor the circulation of plasmids among bacterial strains of different sources or to track the horizontal transmission of AMR genes among the *Enterobacteriaceae* (Carattoli et al., 2005).

In the present study, IncFIIA plasmid was most prevalent (28.7%). IncF has been previously identified as the most common Inc group in *Enterobacteriaceae* from different sources (Yang et al., 2015), The particular interest because several virulence plasmids belong to the IncF plasmid (Silva et al., 2017). The gene-addiction systems (e.g., *relB/relE*) are usually located on IncF plasmids that are also contributed to plasmid dissemination by horizontal transfer (Carattoli, 2007).

Interestingly, IncF plasmids (n=9) are not in a homogenous group (Table 8) which is similar to previous study (Villa et al., 2010; Yang et al., 2015). Among identical IncF-Replicon sequence types, we identified plasmid carrying *qnrS* in *Salmonella* Blegdam and the plasmid was defined as S1:A-:B22 on FAB formula basis. This phenomenon has been previously detected in *Salmonella* Enteritidis from human isolate in Italy (Villa et al., 2010). of which only one isolate (No. 88, serovar Weltevreden) was MDR. Another MDR *Salmonella* isolate carrying IncF plasmid, (No. 42, serovar Othmarschen), was not identified by FAB formula. The S3:A-:B- plasmid was observed in a serovar Bardo (No. 109) and was previously found in the human isolate from France (Mohammed et al., 2017). Unfortunately, there are no studies on FAB formula for *Salmonella* available in Thailand and therefore, the comparison within the country cannot be made.

IncI1 plasmids are a narrow host range plasmids that are associated with *Enterobacteriaceae* (Johnson et al., 2011). Recently, IncI1 are frequency identified in

E. coli and *Salmonella* serotypes of animals origin such as poultry and cattle and shown responsible for the spread of resistance determinants (Carattoli et al., 2018). IncI1 plasmids are well known to carry **β**-lactamase genes (Ben Sallem et al., 2014; Smith et al., 2015; Xia et al., 2017). However, 5 IncI1 plasmids obtained did not carry any **β**-lactamase genes tested. They may carry other **β**-lactamase genes that were not included in this study.

Among identical IncI1 sequence types (ST) in this study, we identified plasmid carrying *qnrS* in *Salmonella* Blegdam and the plasmid was assigned to ST136. The IncI1 ST136 has been previously detected in *Salmonella* Enteritidis and found to co-carriage with IncF plasmid from human isolate in Italy (Villa et al., 2010). The IncI1 ST60 was observed in a serovar Malaka (No. 63) and was previously found in *Salmonella* Virchow from human isolate in Germany (Smith et al., 2015). Another IncI1 plasmid from serovar Weltevreden (No. 5) was assigned to IncI1 ST101 and was previously found in *E. coli* isolate from poultry in Colombia (Castellanos et al., 2017).

Two isolates had a unique combination of alleles (serovars Typhimurium, No. 85; *repI1*, 2; *ardA*, 7; *trbA*, 4; *sogS*, 1; *pilL*, 2 and serovars Anatum No. 101; *repI1*, 1; *ardA*, 2; *trbA*, 20; *sogS*, 6; *pilL*, 3), but non-matching to pMLST database. They were classified as “non-typeable” (Table 9.) The serovars Anatum No.101 carried both IncI1 and IncHI1 plasmids and exhibited MDR phenotype. The emergence of resistant bacterial strains was usually originated from clinical isolates, especially *E. coli* and *Salmonella* that usually carry IncI1 plasmids with multiple virulence and resistance gene associated with MGEs (Accogli et al., 2013; Wong et al., 2015). Distribution of such plasmids may promote distribution of resistant *Salmonella* with increasing virulence.

IncHI1 have been previously suggested to be associated with resistance to ampicillin (bla_{TEM}), chloramphenicol (*catA1*), streptomycin (*strA-strB*), sulfamethoxazole (*sul1* and *sul2*), tetracycline (*tetA*), and trimethoprim (*dfrA1*) in *Salmonella* (Kubasova et al., 2016). In this study, *Salmonella* isolates carrying IncHI1 plasmids (serovar Biafra no. 100, serovar Anatum no. 101 serovar Anatum no. 102 serovar Anatum no. 103) were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracyclines, and trimethoprim, in agreement with previously reports in other regions including Vietnam,

Kenya, and China (Wain et al., 2003; Breiman et al., 2012; Yan et al., 2016). These observations indicate that IncHI1 plasmids are widely distributed and may be horizontally transferred.

Based on the HI1 pMLST scheme, 4 HI1 plasmids were assigned to ST16 that is a novel ST and a major plasmid ST among the isolates in this study. The new HI1 plasmids pMLST type identified in this study may be a result of mutation(s) in the alleles. It is possible that these plasmids may provide a new lineage of HI1 plasmids, deviating from the HI1 plasmid (Phan et al., 2009). The novel ST16 plasmids may evolve via accumulation of point mutations and/or acquisition of resistance elements. Previous studies showed that IncHI1 plasmids were mostly observed in MDR *S.* Typhi (Wain et al., 2003; Holt et al., 2011) and suggested that the emergence of MDR *Salmonella* carrying IncHI1 plasmids happened by acquisition of the plasmids from other *Salmonella enterica* strains or from other *Enterobacteriaceae* through horizontal gene transfer.

The plasmids identified in this study showed diversity and similarities in some replicon types. Despite the plasmids had shared homologous sequence with previously sequenced plasmids, there were also novel (i.e., plasmid ST16) and un-typeable sequences. The previous study has been shown that some resistance genes was associated with increasing prevalence of certain virulence genes (Matsumura et al., 2013), suggesting that use of antibiotic may co-select for virulence genes, and create a possible linkage of virulence and resistance determinants on genetic elements such as integrons and transposons may exist (Lay et al., 2012). Nevertheless, further comparative analysis of plasmids and resistance genes will further advance the knowledge of how AMR in companion animals spread *Salmonella* as well as in humans, and livestock environment (McMillan et al., 2019).

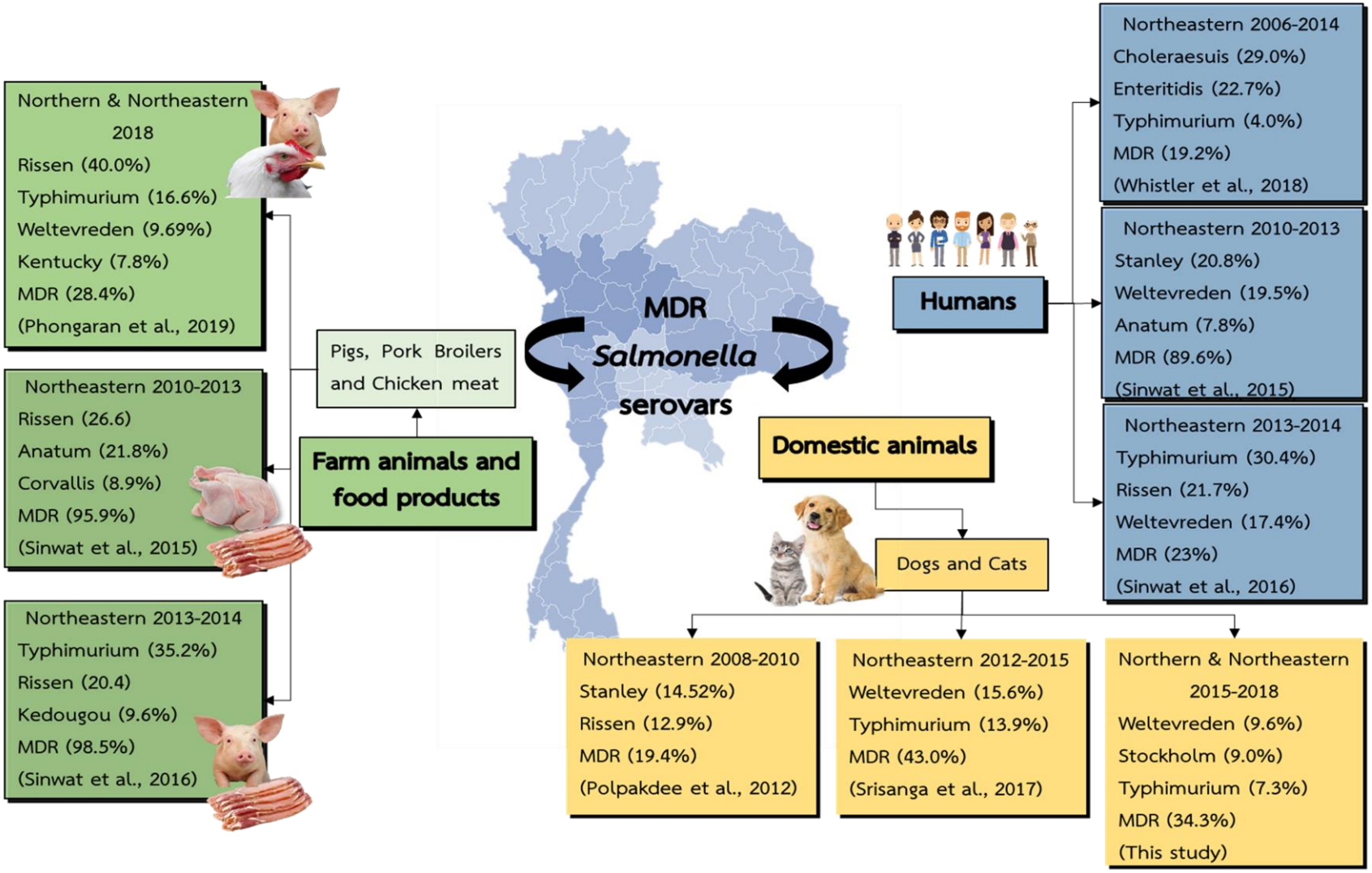


Figure 7: MDR *Salmonella* serovars isolated from different sources in Northern and Northeastern Thailand from 2006 to *2018*

CHAPTER VI CONCLUSION

In conclusion, the objectives of this study were achieved. The major findings are as follows:

1. A variety of *Salmonella* serovars were identified among the isolates from dogs and cats in this collection. Serovar Weltevreden, Stockholm and Typhimurium were common.
2. The majority of *Salmonella* were resistant to antimicrobial agents.
3. Only one ESBL producer was obtained from a dog. The ESBL-positive serovar Stockholm harbored $bla_{\text{CTX-M-55}}$ of CTX-M group 1 and $bla_{\text{TEM-1}}$.
4. None of the isolates were positive to *mcr-1-5*.
5. The *qnrS* gene was detected in 18 *Salmonella* isolates from dogs.
6. Plasmids with a variety of Inc groups can be identified in *Salmonella enterica* in Thailand. Five Inc groups (i.e. IncA/C, IncFIIA, IncHI1, IncI1, and IncN) were detected, of which IncFIIA was most prevalent.
7. The S1:A-:B- was most common among AMR *Salmonella* carrying IncF plasmid replicon alleles.
8. The HI1 plasmid ST 16 was most common.

Applications:

The results obtained from this study can be applied as follows:

1. The information on the occurrence and distribution of AMR could be used as part of national AMR surveillance.
2. The results could be used to support the development guidelines on the antimicrobials use in companion animals.
3. Data can be used in combination with data of food animals, foods, and humans to explain the linkage of AMR using One Health concept.

Recommendations

The results highlight the important role of household dogs and cats as the potential sources of resistant *Salmonella enterica*. Suggestions can be made as follows:

1. It is important for all veterinarians and pet owners to practice prudent use of antimicrobial agents and good hygiene when contact with pet animals.
2. Antimicrobial stewardship strategies should be practiced in animal hospitals.
3. It is strongly recommended that bacteria originating from dogs, cats, and other pet animals should be included in AMR monitoring and surveillance programs.

Further investigations

To date, data and activity on AMR related to companion animals is still limited. Actions need to be taken to face this overwhelming issue. Further investigations are warranted as follows:

1. Additional studies with larger sample size are suggested.
2. Association between resistance and virulence genes in *Salmonella* isolate from dogs and cats should be determined.
3. AMR studies in pathogenic *Salmonella* in other pets and wildlifes should be conducted.
4. Data on *Salmonella enterica* isolated from dogs and cats and their serovar relatedness should be produced.
5. Study on other mobile genetic elements and transfer of AMR determinants in *Salmonella enterica* should be performed.
6. R plasmids obtained from this study can be used for further studies by next generation sequencing.

REFERENCES

Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, White DG, Hasman H, Sørensen G, Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, Angulo FJ and Gerner-Smidt P 2007. International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerg Infect Dis. 13(5): 726-731.

Accogli M, Fortini D, Giufrè M, Graziani C, Dolejska M, Carattoli A and Cerquetti M 2013. IncI1 plasmids associated with the spread of CMY-2, CTX-M-1 and SHV-12 in *Escherichia coli* of animal and human origin. Clin Microbiol Infect. 19(5): 238-240.

Ahamed Riyaaz AA, Perera V, Sivakumaran S and de Silva N 2018. Typhoid Fever due to Extended Spectrum β-Lactamase-Producing *Salmonella enterica* Serovar Typhi: A Case Report and Literature Review. Case Rep Infect Dis. 2018: 4610246.

Anjum MF, Duggett NA, AbuOun M, Randall L, Nunez-Garcia J, Ellis RJ, Rogers J, Horton R, Brena C, Williamson S, Martelli F, Davies R and Teale C 2016. Colistin resistance in *Salmonella* and *Escherichia coli* isolates from a pig farm in Great Britain. J Antimicrob Chemother. 71(8): 2306-2313.

Balasubramanian R, Im J, Lee JS, Jeon HJ, Mogeni OD, Kim JH, Rakotozandrindrainy R, Baker S and Marks F 2019. The global burden and epidemiology of invasive non-typhoidal *Salmonella* infections. Hum Vaccin Immunother. 15(6): 1421-1426.

Barton MD 2014. Impact of antibiotic use in the swine industry. Curr Opin Microbiol. 19: 9-15.

Batchelor M, Hopkins K, Threlfall EJ, Clifton-Hadley FA, Stallwood AD, Davies RH and Liebana E 2005. bla(CTX-M) genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. J Antimicrob Chemother. 49(4): 1319-1322.

Ben Sallem R, Ben Slama K, Rojo-Bezares B, Porres-Osante N, Jouini A, Klibi N, Boudabous A, Sáenz Y and Torres C 2014. IncI1 Plasmids Carrying $bla_{CTX-M-1}$ or bla_{CMY-2} Genes in *Escherichia coli* from Healthy Humans and Animals in Tunisia. Microb Drug Resist. 20(5): 495-500.

Boerlin P and Reid-Smith RJ 2008. Antimicrobial resistance: its emergence and

transmission. Anim Health Res Rev. 9(2): 115-126.

Breiman RF, Cosmas L, Njuguna H, Audi A, Olack B, Ochieng JB, Wamola N, Bigogo GM, Awiti G, Tabu CW, Burke H, Williamson J, Oundo JO, Mintz ED and Feikin DR 2012. Population-based incidence of typhoid fever in an urban informal settlement and a rural area in Kenya: implications for typhoid vaccine use in Africa. PLoS One. 7(1): 29119.

Brown AC, Grass JE, Richardson LC, Nisler AL, Bicknese AS and Gould LH 2017. Antimicrobial resistance in *Salmonella* that caused foodborne disease outbreaks: United States, 2003-2012. Epidemiol Infect. 145(4): 766-774.

Carattoli A 2007. Evolution of Plasmids and Evolution of Virulence and Antibiotic-Resistance Plasmids. In: Evolutionary Biology of Bacterial and Fungal Pathogens. C. Nombela F. Baquero, G.H. Cassell and J.A. Gutiérrez-Fuentes (ed). American Society of Microbiology.

Carattoli A 2013. Plasmids and the spread of resistance. Int J Med Microbiol. 303(6): 298-304.

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL and Threlfall EJ 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 63(3): 219-228.

Carattoli A, Villa L, Fortini D and García-Fernández A 2018. Contemporary IncI1 plasmids involved in the transmission and spread of antimicrobial resistance in *Enterobacteriaceae*. Plasmid. 102392.

Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F and Hasman H 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. J Antimicrob Chemother. 58(7): 3895-3903.

Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO and Wiedmann M 2019. Identification of Novel Mobilized Colistin Resistance Gene *mcr-9* in a Multidrug-Resistant, Colistin-Susceptible *Salmonella enterica* Serotype Typhimurium Isolate. mBio. 10(3).

Castellanos LR, Donado-Godoy P, León M, Clavijo V, Arevalo A, Bernal JF, Timmerman AJ, Mevius DJ, Wagenaar JA and Hordijk J 2017. High Heterogeneity of

Escherichia coli Sequence Types Harbouring ESBL/AmpC Genes on IncI1 Plasmids in the Colombian Poultry Chain. PLoS One. 12(1): e0170777.

Castro K, Muñoz E, García G, Ramírez J, Valencia G, Basulto G, Manríquez L and Renteria T 2019. Prevalence, risk factors, and identification of *Salmonella* spp. in stray dogs of northwest Mexico. Austral J Vet Sci. 51.

Chen W, Fang T, Zhou X, Zhang D, Shi X and Shi C 2016. IncHI2 Plasmids Are Predominant in Antibiotic-Resistant *Salmonella* Isolates. Front Microbiol. 7: 1566.

Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, Chien MS and Lin TY 2002. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype Choleraesuis. N Engl J Med. 346(6): 413-419.

Chuanchuen R and Padungtod P 2009. Antimicrobial Resistance Genes in *Salmonella enterica* Isolates from Poultry and Swine in Thailand. J Vet Med Sci. 71(10): 1349-1355.

CLSI 2013. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. Vol 33. 3 ed. In: CLSI supplement VET01S. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

CLSI 2014. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. Vol 34. 4 ed. In: CLSI document VET01-A4. Clinical Laboratory Standards Institute, Wayne, PA, USA.

Cordeiro NF, Nabón A, García-Fulgueiras V, Álvez M, Sirok A, Camou T and Vignoli R 2016. Analysis of plasmid-mediated quinolone and oxyimino-cephalosporin resistance mechanisms in *Salmonella enterica* Uruguayan isolates from 2011–2013. J Glob Antimicrob Resist. 6: 165-171.

Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler LH, Windahl U, Vanrompay D and Guardabassi L 2016. Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspectives for Targeted Research and Policy Actions. J Comp Pathol. 155(1 Suppl 1): 27-40.

Davies RH, Lawes JR and Wales AD 2019. Raw diets for dogs and cats: a review, with particular reference to microbiological hazards. J Small Anim Pract. 60(6): 329-339.

Devanga Ragupathi NK, Muthuirulandi Sethuvel DP, Gajendran R, Anandan S, Walia K and Veeraraghavan B 2019. Horizontal Transfer of Antimicrobial Resistance Determinants Among Enteric Pathogens Through Bacterial Conjugation. Curr Microbiol. 76(6): 666-672.

Dhama K, Rajagunalan S, Chakraborty S, Verma AK, Kumar A, Tiwari R and Kapoor S 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. Pak J Biol Sci. 16(20): 1076-1085.

DLD 2019. "Subject: The population of dogs and cats in 2016" (online). Available: http://dcontrol.dld.go.th/dcontrol/index.php/rabies/747-dogpop2016.

Doi Y, Iovleva A and Bonomo RA 2017. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J Travel Med. 24(suppl_1): 44-51.

EFSA E 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 16(12): 5500.

EFSA E 2019. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA J. 17(2): e05598.

Fàbrega A and Vila J 2013. *Salmonella enterica* serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev. 26(2): 308-341.

Fang FC 2015. Fluoroquinolone Resistance in *Salmonella* and the Utility of Pefloxacin Disk Diffusion. J Clin Microbiol. 53(11): 3401-3404.

Ferreira JC, Penha Filho RAC, Kuaye APY, Andrade LN, Berchieri Junior A and Darini ALdC 2018. Identification and characterization of plasmid-mediated quinolone resistance determinants in *Enterobacteriaceae* isolated from healthy poultry in Brazil. Infect Genet Evol. 60: 66-70.

García-Fernández A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A and Carattoli A 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J Antimicrob Chemother. 61(6): 1229-1233.

Garcia-Graells C, De Keersmaecker SCJ, Vanneste K, Pochet B, Vermeersch K, Roosens N, Dierick K and Botteldoorn N 2018. Detection of Plasmid-Mediated Colistin Resistance, *mcr-1* and *mcr-2* Genes, in *Salmonella* spp. Isolated from Food at

Retail in Belgium from 2012 to 2015. Foodborne Pathog Dis. 15(2): 114-117.

Grimont PA and Weill F-X 2007. Antigenic formulae of the *Salmonella* serovars. WHO collaborating centre for reference and research on *Salmonella*. 9: 1-166.

Guardabassi L, Schwarz S and Lloyd DH 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria: Review. J Antimicrob Chemother. 54(2): 321-332.

Hancock SJ, Phan M-D, Peters KM, Forde BM, Chong TM, Yin W-F, Chan K-G, Paterson DL, Walsh TR, Beatson SA and Schembri MA 2017. Identification of IncA/C Plasmid Replication and Maintenance Genes and Development of a Plasmid Multilocus Sequence Typing Scheme. Antimicrob Agents Chemother. 61(2): e01740-01716.

Hasman H, Mevius D, Veldman K, Olesen I and Aarestrup FM 2005. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother. 56(1): 115-121.

Helms M, Ethelberg S and Mølbak K 2005. International *Salmonella* Typhimurium DT104 Infections, 1992-2001. J Emerg Infect Dis. 11(6): 859-867.

Hendriksen RS, Bangtrakulnonth A, Pulsrikarn C, Pornruangwong S, Noppornphan G, Emborg HD and Aarestrup FM 2009. Risk factors and epidemiology of the ten most common *Salmonella* serovars from patients in Thailand: 2002-2007. Foodborne Pathog Dis. 6(8): 1009-1019.

Herikstad H, Motarjemi Y and Tauxe RV 2002. *Salmonella* surveillance: a global survey of public health serotyping. Epidemiol Infect. 129(1): 1-8.

Hoelzer K, Moreno Switt AI and Wiedmann M 2011. Animal contact as a source of human non-typhoidal salmonellosis. J Vet Res. 42(1): 34.

Holt KE, Phan MD, Baker S, Duy PT, Nga TVT, Nair S, Turner AK, Walsh C, Fanning S, Farrell-Ward S, Dutta S, Kariuki S, Weill F-X, Parkhill J, Dougan G and Wain J 2011. Emergence of a globally dominant IncHI1 plasmid type associated with multiple drug resistant typhoid. PLoS Negl Trop Dis. 5(7): 1245.

Hooper DC and Jacoby GA 2015. Mechanisms of drug resistance: quinolone resistance. Ann N Y Acad Sci. 1354(1): 12-31.

Hounmanou YMG, Dalsgaard A, Sopacua TF, Uddin GMN, Leekitcharoenphon P,

Hendriksen RS, Olsen JE and Larsen MH 2020. Molecular Characteristics and Zoonotic Potential of *Salmonella* Weltevreden From Cultured Shrimp and Tilapia in Vietnam and China. Front Microbiol. 11: 1985.

Irfan S, Khan E, Jabeen K, Bhawan P, Hopkins KL, Day M, Nasir A, Meunier D and Woodford N 2015. Clinical isolates of *Salmonella enterica* serovar Agona producing NDM-1 metallo-beta-lactamase: first report from Pakistan. J Clin Microbiol. 53(1): 346-348.

ISO 2002. Microbiology of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* spp. 4 ed. In: ISO 6579:2002(E).

Jacoby GA 2005. Mechanisms of Resistance to Quinolones. Clin Infect Dis. 41(Supplement_2): 120-126.

Jain P, Sudhanthirakodi S, Chowdhury G, Joshi S, Anandan S, Ray U, Mukhopadhyay A and Dutta S 2018. Antimicrobial resistance, plasmid, virulence, multilocus sequence typing and pulsed-field gel electrophoresis profiles of *Salmonella* enterica serovar Typhimurium clinical and environmental isolates from India. PLoS One. 13(12): e0207954.

Johnson TJ, Shepard SM, Rivet B, Danzeisen JL and Carattoli A 2011. Comparative genomics and phylogeny of the IncI1 plasmids: A common plasmid type among porcine enterotoxigenic *Escherichia coli*. Plasmid. 66(3): 144-151.

Karkey A, Thwaites GE and Baker S 2018. The evolution of antimicrobial resistance in *Salmonella* Typhi. Curr Opin Gastroenterol. 34(1): 25-30.

Kiflu B, Alemayehu H, Abdurahaman M, Negash Y and Eguale T 2017. *Salmonella* serotypes and their antimicrobial susceptibility in apparently healthy dogs in Addis Ababa, Ethiopia. BMC Vet Res. 13(1): 134.

Kim JS, Yun YS, Kim SJ, Jeon SE, Lee DY, Chung GT, Yoo CK and Kim J 2016. Rapid Emergence and Clonal Dissemination of CTX-M-15-Producing *Salmonella enterica* Serotype Virchow, South Korea. J Emerg Infect Dis. 22(1): 68-70.

Kubasova T, Cejkova D, Matiasovicova J, Sekelova Z, Polansky O, Medvecky M, Rychlik I and Juricova H 2016. Antibiotic Resistance, Core-Genome and Protein Expression in IncHI1 Plasmids in *Salmonella* Typhimurium. Genome Biol Evol. 8(6): 1661-1671.

Lay KK, Koowattananukul C, Chansong N and Chuanchuen R 2012. Antimicrobial Resistance, Virulence, and Phylogenetic Characteristics of *Escherichia coli* Isolates from Clinically Healthy Swine. Foodborne Pathog Dis. 9(11): 992-1001.

Leonard EK, Pearl DL, Finley RL, Janecko N, Reid-Smith RJ, Peregrine AS and Weese JS 2011. Comparison of antimicrobial resistance patterns of *Salmonella* spp. and *Escherichia coli* recovered from pet dogs from volunteer households in Ontario (2005–2006). J Antimicrob Chemother. 67(1): 174-181.

Leonard F 2014. *Salmonella* infection and carriage: the importance of dogs and their owners. Vet Rec. 174(4): 92.

Lertworapreecha M, Sutthimusik S and Tontikapong K 2012. Antimicrobial Resistance in *Salmonella enterica* Isolated From Pork, Chicken, and Vegetables in Southern Thailand. Jundishapur J Microbiol. 6(1): 36-41.

Lévesque C, Piché L, Larose C and Roy PH 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother. 39(1): 185-191.

Li B, Yang X, Tan H, Ke B, He D, Wang H, Chen Q, Ke C and Zhang Y 2018. Whole genome sequencing analysis of *Salmonella enterica* serovar Weltevreden isolated from human stool and contaminated food samples collected from the Southern coastal area of China. Int J Food Microbiol. 266: 317-323.

Liakopoulos A, Geurts Y, Dierikx CM, Brouwer MSM, Kant A, Wit B, Heymans R, van Pelt W and Mevius DJ 2016. Extended-Spectrum Cephalosporin-Resistant *Salmonella enterica* serovar Heidelberg Strains, the Netherlands. J Emerg Infect Dis. 22(7): 1257-1261.

Liebana E, Garcia-Migura L, Clouting C, Clifton-Hadley FA, Lindsay E, Threlfall EJ, McDowell SW and Davies RH 2002. Multiple genetic typing of *Salmonella enterica* serotype typhimurium isolates of different phage types (DT104, U302, DT204b, and DT49) from animals and humans in England, Wales, and Northern Ireland. J Clin Microbiol. 40(12): 4450-4456.

Lin D, Chen K, Wai-Chi Chan E and Chen S 2015. Increasing prevalence of ciprofloxacin-resistant food-borne *Salmonella* strains harboring multiple PMQR elements but not target gene mutations. Sci Rep. 5: 14754.

Lindblom A, Kk S, Müller V, Öz R, Sandström H, Åhrén C, Westerlund F and Karami N 2019. Interspecies plasmid transfer appears rare in sequential infections with extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. Diagn Microbiol Infect Dis. 93(4): 380-385.

Ling J, Chan E, Lam A and Cheng A 2003. Mutations in topoisomerase genes of fluoroquinolone-resistant *Salmonellae* in Hong Kong. J Antimicrob Chemother. 47(11): 3567-3573.

Liu G, Bogaj K, Bortolaia V, Olsen JE and Thomsen LE 2019. Antibiotic-Induced, Increased Conjugative Transfer Is Common to Diverse Naturally Occurring ESBL Plasmids in *Escherichia coli*. Front Microbiol. 10: 2119.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH and Shen J 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 16(2): 161-168.

Lowden P, Wallis C, Gee N and Hilton A 2015. Investigating the prevalence of *Salmonella* in dogs within the Midlands region of the United Kingdom. BMC Vet Res. 11: 239.

Lynne AM, Dorsey LL, David DE and Foley SL 2009. Characterisation of antibiotic resistance in host-adapted *Salmonella enterica*. Int J Antimicrob Agents. 34(2): 169-172.

Ma Y, Li M, Xu X, Fu Y, Xiong Z, Zhang L, Qu X, Zhang H, Wei Y, Zhan Z, Chen Z, Bai J, Liao M and Zhang J 2018. High-levels of resistance to quinolone and cephalosporin antibiotics in MDR-ACSSuT *Salmonella enterica* serovar Enteritidis mainly isolated from patients and foods in Shanghai, China. Int J Food Microbiol. 286: 190-196.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT and Monnet DL 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol

Infect. 18(3): 268-281.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A and Hoekstra RM 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis. 50(6): 882-889.

Matsumura Y, Yamamoto M, Nagao M, Ito Y, Takakura S and Ichiyama S 2013. Association of Fluoroquinolone Resistance, Virulence Genes, and IncF Plasmids with Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Sequence Type 131 (ST131) and ST405 Clonal Groups. J Antimicrob Chemother. 57(10): 4736-4742.

McMillan EA, Gupta SK, Williams LE, Jové T, Hiott LM, Woodley TA, Barrett JB, Jackson CR, Wasilenko JL, Simmons M, Tillman GE, McClelland M and Frye JG 2019. Antimicrobial Resistance Genes, Cassettes, and Plasmids Present in *Salmonella enterica* Associated With United States Food Animals. Front Microbiol. 10: 832.

Michael GB and Schwarz S 2016. Antimicrobial resistance in zoonotic nontyphoidal *Salmonella*: an alarming trend? Clin Microbiol Infect. 22(12): 968-974.

Miró E, Vergés C, García I, Mirelis B, Navarro F, Coll P, Prats G and Martínez-Martínez L 2004. Resistance to quinolones and beta-lactams in *Salmonella enterica* due to mutations in topoisomerase-encoding genes, altered cell permeability and expression of an active efflux system. Enferm Infecc Microbiol Clin. 22(4): 204-211.

Mohammed M, Le Hello S, Leekitcharoenphon P and Hendriksen R 2017. The invasome of *Salmonella* Dublin as revealed by whole genome sequencing. BMC Infect Dis. 17(1): 544.

Murase T, Ozaki H, Phuektes P and Angkititrakul S 2018. Genotypic and phenotypic characterization of *Salmonella enterica* subsp. enterica serovar Typhimurium monophasic variants isolated in Thailand and Japan. J Vet Med Sci. 80(12): 1839-1846.

Nadimpalli M, Fabre L, Yith V, Sem N, Gouali M, Delarocque-Astagneau E, Sreng N and Le Hello S 2019. CTX-M-55-type ESBL-producing *Salmonella enterica* are emerging among retail meats in Phnom Penh, Cambodia. J Antimicrob Chemother. 74(2): 342-348.

Novick RP 1987. Plasmid incompatibility. Microbiol Rev. 51(4): 381-395.

Nuanmuang N and Kummasook A 2018. Prevalence and Antimicrobial Resistance of *Salmonella* in Minced Pork from Retail Shops around the University of Phayao, Thailand. Warasan Witthayasat Mahawitthayalai Naresuan. 26(4): 9-16.

Osman D and Cavet JS 2011. Metal Sensing in *Salmonella*: Implications for Pathogenesis. In: Advances in microbial physiology. Robert K. Poole (ed). Academic Press. 175-232.

Palzkill T 2018. Structural and Mechanistic Basis for Extended-Spectrum Drug-Resistance Mutations in Altering the Specificity of TEM, CTX-M, and KPC β-lactamases. Front Mol Biosci. 5: 16.

Park CH, Robicsek A, Jacoby GA, Sahm D and Hooper DC 2006. Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. J Antimicrob Chemother. 50(11): 3953-3955.

Patchanee P, Tansiricharoenkul K, Buawiratlert T, Wiratsudakul A, Angchokchatchawal K, Yamsakul P, Yano T, Boonkhot P, Rojanasatien S and Tadee P 2016. *Salmonella* in pork retail outlets and dissemination of its pulsotypes through pig production chain in Chiang Mai and surrounding areas, Thailand. Prev Vet Med. 130: 99-105.

Phan M-D, Kidgell C, Nair S, Holt KE, Turner AK, Hinds J, Butcher P, Cooke FJ, Thomson NR, Titball R, Bhutta ZA, Hasan R, Dougan G and Wain J 2009. Variation in *Salmonella enterica* serovar typhi IncHI1 plasmids during the global spread of resistant typhoid fever. J Antimicrob Chemother. 53(2): 716-727.

Phongaran D, Khang-Air S and Angkititrakul S 2019. Molecular epidemiology and antimicrobial resistance of *Salmonella* isolates from broilers and pigs in Thailand. Veterinary world. 12(8): 1311-1318.

Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK and Tuladhar NR 2006. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int J Infect Dis. 10(6): 434-438.

Polpakdee A, Angkititra S, Suksawat F, Sparagano O and Kanistanon K 2012. Epidemiology and Antimicrobial Resistance of *Salmonella* sp. Isolated from

Dogs and Cats in Northeastern Thailand. J Anim Vet Adv. 11: 618-621.

Ponce E, Khan AA, Cheng CM, Summage-West C and Cerniglia CE 2008. Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiol. 25(1): 29-35.

Prestinaci F, Pezzotti P and Pantosti A 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 109(7): 309-318.

Pungpian C, Sinwat N, Angkititrakul S, Prathan R and Chuanchuen R 2020. Presence and Transfer of Antimicrobial Resistance Determinants in *Escherichia coli* in Pigs, Pork, and Humans in Thailand and Lao PDR Border Provinces. Microb Drug Resist.

Queenan K, Häsler B and Rushton J 2016. A One Health approach to antimicrobial resistance surveillance: is there a business case for it. Int J Antimicrob Agents. 48(4): 422-427.

Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A and Hendriksen RS 2018. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* for surveillance purposes. Euro Surveill. 23(6): 17-672.

Reed TAN, Krang S, Miliya T, Townell N, Letchford J, Bun S, Sar B, Osbjer K, Seng S, Chou M, By Y, Vanchinsuren L, Nov V, Chau D, Phe T, de Lauzanne A, Ly S and Turner P 2019. Antimicrobial resistance in Cambodia: a review. Int J Infect Dis. 85: 98-107.

Reimschuessel R, Grabenstein M, Guag J, Nemser SM, Song K, Qiu J, Clothier KA, Byrne BA, Marks SL, Cadmus K, Pabilonia K, Sanchez S, Rajeev S, Ensley S, Frana TS, Jergens AE, Chappell KH, Thakur S, Byrum B, Cui J, Zhang Y, Erdman MM, Rankin SC, Daly R, Das S, Ruesch L, Lawhon SD, Zhang S, Baszler T, Diaz-Campos D, Hartmann F and Okwumabua O 2017. Multilaboratory Survey To Evaluate *Salmonella* Prevalence in Diarrheic and Nondiarrheic Dogs and Cats in the United States between 2012 and 2014. J Clin Microbiol. 55(5): 1350-1368.

Robicsek A, Jacoby GA and Hooper DC 2006. The worldwide emergence of plasmid-

mediated quinolone resistance. Lancet Infect Dis. 6(10): 629-640.

Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ and Hordijk J 2018. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. J Antimicrob Chemother. 73(5): 1121-1137.

Silva C, Calva E and Maloy S 2014. One Health and Food-Borne Disease: *Salmonella* Transmission between Humans, Animals, and Plants. Microbiol Spectr. 2(1): Oh-0020-2013.

Silva C, Puente JL and Calva E 2017. *Salmonella* virulence plasmid: pathogenesis and ecology. Pathogens and Disease. 75(6).

Sinwat N, Angkittitrakul S and Chuanchuen R 2015. Characterization of Antimicrobial Resistance in *Salmonella enterica* Isolated from Pork, Chicken Meat, and Humans in Northeastern Thailand. Foodborne Pathog Dis. 12(9): 759-765.

Sinwat N, Angkittitrakul S, Coulson KF, Pilapil F, Meunsene D and Chuanchuen R 2016. High prevalence and molecular characteristics of multidrug-resistant *Salmonella* in pigs, pork and humans in Thailand and Laos provinces. Int J Med Microbiol. 65(10): 1182-1193.

Sirichote P, Bangtrakulnonth A, Tianmanee K, Unahalekhaka A, Oulai A, Chittaphithakchai P, Kheowrod W and Hendriksen RS 2010. Serotypes and antimicrobial resistance of *Salmonella enterica* ssp in central Thailand, 2001-2006. Southeast Asian J Trop Med Public Health. 41(6): 1405-1415.

Smith H, Bossers A, Harders F, Wu G, Woodford N, Schwarz S, Guerra B, Rodríguez I, van Essen-Zandbergen A, Brouwer M and Mevius D 2015. Characterization of epidemic IncI1-Iγ plasmids harboring ambler class A and C genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. J Antimicrob Chemother. 59(9): 5357-5365.

Srisanga S, Angkititrakul S, Sringam P, Le Ho PT, T Vo AT and Chuanchuen R 2017. Phenotypic and genotypic antimicrobial resistance and virulence genes of *Salmonella enterica* isolated from pet dogs and cats. J Vet Sci. 18(3): 273-281.

Stephenson S, Brown P and Wilks M 2009. P75 Emergence of *qnr*-mediated quinolone resistance among *Enterobacteriaceae* in Jamaica. Int J Antimicrob Agents. 34:

S51.

Stokes HW and Gillings MR 2011. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiol Rev. 35(5): 790-819.

Su LH, Chiu CH, Chu C and Ou JT 2004. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin Infect Dis. 39(4): 546-551.

Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH and Haq QMR 2018. Antibiotics, Resistome and Resistance Mechanisms: A Bacterial Perspective. Front Microbiol. 9: 2066.

Szmolka A and Nagy B 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Front Microbiol. 4: 258.

Tadee P, Boonkhot P, Pornruangwong S and Patchanee P 2015. Comparative phenotypic and genotypic characterization of *Salmonella* spp. in pig farms and slaughterhouses in two provinces in northern Thailand. PLoS One. 10(2): 116581.

Thomas CM and Nielsen KM 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol. 3(9): 711-721.

Thong KL, Goh YL, Radu S, Noorzaleha S, Yasin R, Koh YT, Lim VKE, Rusul G and Puthucheary SD 2002. Genetic diversity of clinical and environmental strains of *Salmonella enterica* serotype Weltevreden isolated in Malaysia. J Clin Microbiol. 40(7): 2498-2503.

Tindall BJ, Grimont PA, Garrity GM and Euzeby JP 2005. Nomenclature and taxonomy of the genus *Salmonella*. Int J Syst Evol Microbiol. 55(Pt 1): 521-524.

Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y and Spencer J 2019. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J Mol Biol. 431(18): 3472-3500.

Trongjit S, Angkititrakul S, Tuttle RE, Poungseree J, Padungtod P and Chuanchuen R 2017. Prevalence and antimicrobial resistance in *Salmonella enterica* isolated from broiler chickens, pigs and meat products in Thailand-Cambodia border provinces. Microbiol Immunol. 61(1): 23-33.

Villa L, García-Fernández A, Fortini D and Carattoli A 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob

Chemother. 65(12): 2518-2529.

Virolle C, Goldlust K, Djermoun S, Bigot S and Lesterlin C 2020. Plasmid Transfer by Conjugation in Gram-Negative Bacteria: From the Cellular to the Community Level. Genes (Basel). 11(11): 1239.

Von Wintersdorff CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, van Alphen LB, Savelkoul PHM and Wolffs PFG 2016. Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. Front Microbiol. 7(173).

Wain J, Diem Nga LT, Kidgell C, James K, Fortune S, Song Diep T, Ali T, O Gaora P, Parry C, Parkhill J, Farrar J, White NJ and Dougan G 2003. Molecular analysis of IncHI1 antimicrobial resistance plasmids from *Salmonella* serovar Typhi strains associated with typhoid fever. J Antimicrob Chemother. 47(9): 2732-2739.

Wang J, Huang X-Y, Xia Y-B, Guo Z-W, Ma Z-B, Yi M-Y, Lv L-C, Lu P-L, Yan J-C, Huang J-W, Zeng Z-L and Liu J-H 2018. Clonal Spread of *Escherichia coli* ST93 Carrying *mcr-1*-Harboring IncN1-IncHI2/ST3 Plasmid Among Companion Animals, China. Front Microbiol. 9: 2989.

Weill FX, Lailler R, Praud K, Kérouanton A, Fabre L, Brisabois A, Grimont PA and Cloeckaert A 2004. Emergence of extended-spectrum-beta-lactamase (CTX-M-9)-producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. J Clin Microbiol. 42(12): 5767-5773.

Whistler T, Sapchookul P, McCormick DW, Sangwichian O, Jorakate P, Makprasert S, Jatapai A, Naorat S, Surin U, Koosakunwat S, Supcharassaeng S, Piralam B, Mikoleit M, Baggett HC, Rhodes J and Gregory CJ 2018. Epidemiology and antimicrobial resistance of invasive non-typhoidal Salmonellosis in rural Thailand from 2006-2014. PLoS Negl Trop Dis. 12(8): e0006718.

WHO 2019. Global Antimicrobial Resistance Surveillance System (GLASS): molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System. World Health Organization, Geneva.

Wong MH-y, Kan B, Chan EW-c, Yan M and Chen S 2015. IncI1 Plasmids Carrying Various blaCTX-M Genes Contribute to Ceftriaxone Resistance in *Salmonella enterica*

Serovar Enteritidis in China. J Antimicrob Chemother. 60(2): 982-989.

Wong MH, Chan EW, Liu LZ and Chen S 2014. PMQR genes *oqxAB* and *aac(6')Ib-cr* accelerate the development of fluoroquinolone resistance in *Salmonella* Typhimurium. Front Microbiol. 5: 512.

Wu X, Angkititrakul S, L Richards A, Pulsrikarn C, Khaengair S, Keosengthong A, Siriwong S and Suksawat F 2020. Risk of Antimicrobial Resistant Non-Typhoidal *Salmonella* during Asymptomatic Infection Passage between Pet Dogs and Their Human Caregivers in Khon Kaen, Thailand. Antibiotics (Basel). 9(8): 477.

Xia L, Liu Y, Xia S, Kudinha T, Xiao S-N, Zhong N-S, Ren G-S and Zhuo C 2017. Prevalence of ST1193 clone and IncI1/ST16 plasmid in *E-coli* isolates carrying bla(CTX-M-55) gene from urinary tract infections patients in China. Sci Rep. 7: 44866.

Xu Y, Zhong L-L, Srinivas S, Sun J, Huang M, Paterson DL, Lei S, Lin J, Li X, Tang Z, Feng S, Shen C, Tian G-B and Feng Y 2018. Spread of MCR-3 Colistin Resistance in China: An Epidemiological, Genomic and Mechanistic Study. EBioMedicine. 34: 139-157.

Yamane K, Wachino J-i, Suzuki S, Kimura K, Shibata N, Kato H, Shibayama K, Konda T and Arakawa Y 2007. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. J Antimicrob Chemother. 51(9): 3354-3360.

Yan M, Li X, Liao Q, Li F, Zhang J and Kan B 2016. The emergence and outbreak of multidrug-resistant typhoid fever in China. Emerg Microbes Infect. 5(6): 62.

Yang Q-E, Sun J, Li L, Deng H, Liu B-T, Fang L-X, Liao X-P and Liu Y-H 2015. IncF plasmid diversity in multi-drug resistant *Escherichia coli* strains from animals in China. Front Microbiol. 6: 964.

Yoon K-B, Song B-J, Shin M-Y, Lim H-C, Yoon Y-H, Jeon D-Y, Ha H, Yang S-I and Kim J-B 2017. Antibiotic Resistance Patterns and Serotypes of *Salmonella* spp. Isolated at Jeollanam-do in Korea. Osong Public Health Res Perspect. 8(3): 211-219.

Yutin N 2013. Horizontal Gene Transfer. In: Brenner's Encyclopedia of Genetics (Second Edition). Stanley Maloy and Kelly Hughes (eds). San Diego: Academic Press. 530-532.

Zhang X-F, Doi Y, Huang X, Li H-Y, Zhong L-L, Zeng K-J, Zhang Y-F, Patil S and Tian G-B 2016. Possible Transmission of *mcr-1*-Harboring *Escherichia coli* between Companion Animals and Human. Emerg Infect Dis. 22(9): 1679-1681.

APPENDIX A

Bacterial Growth Media

1. Muller Hinton Agar (MHA) (Difco™, MD, USA)
    - Beef Extract Powder 2.0 g
    - Acid Digest of Casien 17.5 g
    - Starch 1.5 g
    - Agar 17.0 g
2. Luria-Bertani Agar (LB) (Difco™, MD, USA)
    - Trptone 10.0 g
    - Yeast Ectract 5.0 g
    - Sodium chloride 5.0 g
    - Agar 15.0 g
3. Swarm Agar
    - Brain Heart Infusion Agar (Difco™, MD, USA) 19.0 g
    - Tryptose 5.0 g
    - Agar 7.5 g
    - Distilled deionized water 1,000.0 ml

Chemicals and Antimicrobial agents

1. 50X TAE (Tris-Acetate Buffer)
    - Tris base 242.0 g
    - Acetic acid 57.1 g
    - 0.5M EDTA pH 8.0 100.0 ml
    - Distilled water 1,000.0 ml

2. 0.5M EDTA, pH 8.0
    - Disodium ethylene diamine tetraacetate. $2H_2O$ 121.1 g
    - Distilled deionized water 800.0 ml

3. Agarose gel (Sigma-Aldish®)
    - Agarose (Ultra-pure) 1.5 g
    - 1X TAE Buffer 100.0 ml

Other chemicals

- TE buffer (Tris 10mM and EDTA 1 mM)
- NaOH (0.2M)
- DNA marker (Thermo Scientific™ DNA ladder)
- Loading Dye (Thermo Scientific™ Tristrack DNA Loading Dye)

APPENDIX B

Antimicrobial agents

Antibiotics	Solvents	Concentrations range (µg/ml)
Ampicillin	Distilled water	0.25 – 1,024
Ceftriaxone	Distilled water	0.015625 – 8
Chloramphenicol	95% ethanol	1 – 512
Ciprofloxacin	0.1 N NaOH	0.015625 – 64
Colistin	Distilled water	0.25 - 64
Levofloxacin	Acetic acid	0.0625 – 64
Gentamicin	Distilled water	0.0125 – 64
Streptomycin	Distilled water	1 – 1,024
Sulfamethoxazole	1 N NaOH	2 – 2,048
Tetracycline	70% ethanol	0.0625 - 256
Trimethoprim	Dimethylacetamide	0.25 – 1,024

Table: *Salmonella* serovars isolated from dogs and cats in Northern Thailand (n=36)

Serovars	Northern (n=36)									
	Chiang Mai		Chiang Rai		Lampang		Mae Hong Son		Nan	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Aberdeen	-	-	-	-	-	-	-	-	-	-
Agona	2(5.6)	-	-	-	-	-	-	-	-	-
Amberstra	-	-	-	-	-	-	-	-	-	-
Aminatu	-	-	-	-	-	-	-	-	-	-
Amounderbess	-	-	-	-	-	-	-	-	-	-
Amsterdam	-	-	-	-	-	-	-	-	-	-
Anatum	-	-	-	-	-	-	-	-	-	-
Athinai	-	-	-	-	-	-	-	-	-	-
Bardo	-	-	-	-	-	-	-	-	-	-
Biafra	-	-	-	-	-	-	-	-	-	-
Blegdam	-	-	-	-	-	-	-	-	-	-
Braenderup	-	-	-	-	-	-	-	-	-	-
Bsilla	1(2.8)	-	-	-	-	-	-	-	-	-
Bury	-	-	-	-	-	-	-	-	-	-
Chincol	-	-	-	-	-	-	-	-	-	-
Choleraesuis	-	-	1(2.8)	-	-	-	-	-	-	-
Clanvillian	-	-	-	-	-	-	-	-	-	-
Dallgow	-	-	-	-	-	-	-	-	-	-
Damman	-	-	-	-	-	-	-	-	-	-
Derby	-	-	-	-	-	-	-	-	-	-

Table: *Salmonella* serovars isolated from dogs and cats in Northern Thailand (n=36) (continued)

Serovars	Northern (n=36)									
	Chiang Mai		Chiang Rai		Lampang		Mae Hong Son		Nan	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Dublin	-	-	-	-	-	-	-	-	-	-
Eastbourne	-	-	1(2.8)	-	-	-	-	-	-	-
Eingedi	1(2.8)	-	-	-	-	-	-	-	-	-
Enteritidis	1(2.8)	-	1(2.8)	-	-	-	1(2.8)	-	-	-
Farsta	-	-	-	-	-	-	-	-	-	-
Frankfurt	-	-	-	-	-	-	-	-	-	-
Galil	-	-	-	-	-	-	-	-	-	-
Give	-	-	-	-	-	-	-	-	-	-
Hadar	-	-	-	-	-	-	-	-	-	-
Hill	-	-	-	-	-	-	-	-	-	-
Hissar	-	-	-	-	-	-	-	-	-	-
Hvittingdoss	-	-	-	-	-	-	-	-	-	-
Kedougou	-	-	-	-	-	-	-	-	-	-
Kingston	-	-	-	-	-	-	-	-	-	-
Kisangami	-	-	-	-	-	-	-	-	-	-
Kisii	-	-	-	-	-	-	-	-	-	-
Lekke	-	-	-	-	1(2.8)	-	-	-	-	-
Lomita	1(2.8)	-	-	-	-	-	-	-	-	-
Madras	-	-	-	-	-	-	-	-	1(2.8)	-
Malaka	-	-	-	-	-	-	-	-	-	-

Table: *Salmonella* serovars isolated from dogs and cats in Northern Thailand (n=36) (continued)

Serovars	Northern (n=36)									
	Chiang Mai		Chiang Rai		Lampang		Mae Hong Son		Nan	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Megumeri	-	-	-	-	-	-	-	-	-	-
Menden	1(2.8)	-	1(2.8)	-	-	-	-	-	-	-
Moers	-	-	-	-	-	-	-	-	-	-
Mokola	-	-	-	-	-	-	-	-	-	-
Montevideo	-	-	-	-	-	-	-	-	-	-
Nyborg	-	-	-	-	-	-	-	-	-	-
Onarimon	-	-	-	-	-	-	-	-	-	-
Onireke	-	-	-	-	-	-	-	-	-	-
Oslo	-	-	-	-	-	-	-	-	-	-
Othmarschen	-	-	-	-	-	-	-	-	1(2.8)	-
Parathyphi B	1(2.8)	-	-	1(2.8)	-	-	-	-	-	-
Regent	-	-	-	-	-	-	-	-	-	-
Ridge	-	-	-	-	-	-	-	-	-	-
Rissen	-	-	-	-	-	-	-	-	-	-
Ruzizi	-	-	-	-	-	-	-	-	-	-
Saloniki	-	-	-	-	-	-	-	-	-	-
Saphra	-	-	-	-	-	-	-	-	-	-
Seremban	1(2.8)	-	1(2.8)	-	-	-	-	-	-	-
Stanley	1(2.8)	-	-	-	-	-	-	-	-	-
Stockholm	1(2.8)	-	2(5.6)	-	2(5.6)	-	-	-	-	-

Table: *Salmonella* serovars isolated from dogs and cats in Northern Thailand (n=36) (continued)

Serovars	Northern (n=36)									
	Chiang Mai		Chiang Rai		Lampang		Mae Hong Son		Nan	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Stuttgart	-	-	-	-	-	-	-	-	-	-
Tees	-	-	-	-	-	-	-	-	-	-
Tilburg	-	-	1(2.8)	-	-	-	-	-	-	-
Tokoin	-	-	-	-	-	-	-	-	-	-
Truvo	-	-	-	-	-	-	-	-	-	-
Typhimurium	-	-	-	-	-	-	-	-	-	-
Uppsala	-	-	-	-	2(5.6)	-	-	-	-	-
Utah	-	-	-	-	-	-	-	-	-	-
Vilvoorde	-	-	-	-	-	-	-	-	-	-
Virchow	-	-	-	-	-	-	-	-	-	-
Weltevreden	-	-	-	-	2(5.6)	-	-	-	-	-
Westafrica	-	-	-	-	-	-	-	-	-	-
Weston	-	-	-	1(2.8)	-	-	-	-	-	-
Wimborne	-	-	-	-	-	-	-	-	-	-
Winston	1(2.8)	-	-	-	-	-	-	-	-	-
Worthington	-	-	-	-	-	-	-	-	-	-
Yeerongpilly	-	-	-	-	-	-	-	-	-	-

Table: *Salmonella* serovars isolated from dogs and cats in Northeastern Thailand (n=142)

Serovars	Northeastern (n=142)													
	Bueng Kan		Loei		Mukdaharn		Nakorn Phanom		Nakorn Ratchasima		Nong Khai		Roi Et	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Aberdeen	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Agona	-	-	1(0.7)	-	-	-	-	-	-	-	2(1.4)	-	1(0.7)	-
Amberstra	-	-	-	-	-	-	-	1(0.7)	-	-	-	-	-	-
Aminatu	-	-	3(2.1)	-	-	-	-	-	-	-	-	-	-	-
Amounderbess	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Amsterdam	-	-	-	-	2(1.4)	-	-	-	-	-	-	-	-	-
Anatum	-	-	-	-	3(2.1)	-	-	-	-	-	-	-	-	-
Athinai	-	-	1(0.7)	-	-	-	-	-	-	-	-	-	-	-
Bardo	-	-	1(0.7)	-	1(0.7)	-	-	-	-	-	1(0.7)	-	-	-
Biafra	-	-	-	-	1(0.7)	-	-	-	-	-	1(0.7)	-	-	-
Blegdam	-	-	1(0.7)	-	-	-	-	-	-	-	1(0.7)	-	-	-
Braenderup	1(0.7)	-	-	-	-	-	-	-	-	-	-	-	-	-
Bsilla	-	-	-	-	-	-	-	-	-	1(0.7)	1(0.7)	-	-	-
Bury	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chincol	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Choleraesuis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clanvillian	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Dallgow	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Damman	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Derby	1(0.7)	-	-	-	-	-	-	-	-	-	-	-	-	-

Table: *Salmonella* serovars isolated from dogs and cats in Northeastern Thailand (n=142) (continued)

Serovars	Northeastern (n=142)													
	Bueng Kan		Loei		Mukdaharn		Nakorn Phanom		Nakorn Ratchasima		Nong Khai		Roi Et	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Dublin	-	-	-	-	-	-	-	-	-	-	-	-	1(0.7)	-
Eastbourne	-	-	-	-	-	-	-	-	1(0.7)	-	2(1.4)	-	-	-
Eingedi	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enteritidis	-	-	-	-	-	-	-	-	-	-	2(1.4)	-	-	-
Farsta	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Frankfurt	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Galil	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Give	-	-	-	-	-	-	1(0.7)	-	-	-	-	-	-	-
Hadar	-	-	1(0.7)	-	-	-	-	-	-	-	-	-	-	-
Hill	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Hissar	-	-	-	-	-	-	1(0.7)	-	-	-	-	-	1(0.7)	-
Hvittingdoss	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Kedougou	-	-	-	-	-	-	-	-	-	-	2(1.4)	-	-	-
Kingston	-	-	-	-	-	-	-	-	1(0.7)	-	-	-	-	-
Kisangami	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Kisii	-	-	-	-	-	-	-	-	-	-	-	-	1(0.7)	-
Lekke	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lomita	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Madras	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Malaka	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-

Table: *Salmonella* serovars isolated from dogs and cats in Northeastern Thailand (n=142) (continued)

Serovars	Northeastern (n=142)													
	Bueng Kan		Loei		Mukdaharn		Nakorn Phanom		Nakorn Ratchasima		Nong Khai		Roi Et	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Megumeri	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Menden	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moers	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Mokola	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Montevideo	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nyborg	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Onarimon	-	-	-	-	1(0.7)	-	-	-	-	-	-	-	-	-
Onireke	-	-	-	-	-	-	-	-	-	-	2(1.4)	-	-	-
Oslo	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Othmarschen	-	-	-	-	-	-	-	-	-	-	4(2.8)	-	-	-
Parathyphi B	1(0.7)	-	-	-	-	-	-	-	-	-	1(0.7)	1(0.7)	-	-
Regent	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Ridge	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Rissen	-	-	-	-	1(0.7)	-	1(0.7)	2(1.4)	-	-	4(2.8)	-	2(1.4)	-
Ruzizi	-	-	1(0.7)	-	-	-	-	-	-	-	-	-	-	-
Saloniki	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Saphra	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Seremban	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stanley	-	-	1(0.7)	-	1(0.7)	-	-	-	-	-	2(1.4)	1(0.7)	-	-
Stockholm	-	-	-	-	-	-	4(2.8)	1(0.7)	-	-	-	-	2(1.4)	-

Table: *Salmonella* serovars isolated from dogs and cats in Northeastern Thailand (n=142) (continued)

Serovars	Northeastern (n=142)													
	Bueng Kan		Loei		Mukdaharn		Nakorn Phanom		Nakorn Ratchasima		Nong Khai		Roi Et	
	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)	Dog (%)	Cat (%)
Stuttgart	-	-	-	1(0.7)	-	-	-	-	-	-	-	-	-	-
Tees	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Tilburg	-	-	-	-	1(0.7)	-	-	-	-	-	1(0.7)	-	-	-
Tokoin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Truvo	-	-	-	-	-	-	1(0.7)	-	-	-	-	-	-	-
Typhimurium	3(2.1)	-	-	-	1(0.7)	-	2(1.4)	-	1(0.7)	-	4(2.8)	1(0.7)	1(0.7)	-
Uppsala	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Utah	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Vilvoorde	-	-	-	-	1(0.7)	-	-	-	-	-	-	-	-	-
Virchow	-	-	-	-	-	-	1(0.7)	-	-	-	-	-	-	-
Weltevreden	2(1.4)	-	6(4.2)	1(0.7)	-	-	1(0.7)	-	-	-	6(4.2)	-	-	-
Westafrica	-	-	-	-	-	-	-	-	1(0.7)	-	-	-	-	-
Weston	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wimborne	-	-	-	-	-	-	-	-	-	-	1(0.7)	-	-	-
Winston	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Worthington	-	-	-	-	-	-	-	-	-	-	-	-	1(0.7)	-
Yeerongpilly	-	1(0.7)	-	-	-	-	-	-	-	-	2(1.4)	1(0.7)	-	-

Table: Distribution of MIC value of antibiotics tested in *Salmonella* isolated from dogs and cats, the percentage showed in parenthesis (n=178)

Antimicrobial agents	MIC values (µg/ml)																	
	≤0.0156	**0.03125**	**0.0625**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**≥1024**	**≥2048**
Ampicillin	-	-	-	-	2 (1.1)	45 (25.2)	-	50 (28.1)	11 (6.2)	-	1 (0.6)	1 (0.6)	3 (1.7)	6 (3.4)	22 (12.4)	12 (6.7)	25 (14.0)	-
Ceftriaxone	3 (1.7)	84 (47.2)	66 (37.1)	14 (7.9)	1 (0.6)	-	-	8 (4.5)	-	1 (0.6)	-	-	-	-	-	-	-	-
Chloramphenicol	-	-	-	-	-	-	35 (19.7)	2 (1.1)	68 (38.2)	35 (19.7)	24 (13.5)	2 (1.1)	1 (0.6)	4 (2.2)	6 (3.4)	1 (0.6)	-	-
Ciprofloxacin	121 (68.0)	26 (14.6)	3 (1.7)	7 (3.9)	14 (7.9)	5 (2.8)	2 (1.1)	-	-	-	-	-	-	-	-	-	-	-
Colistin	-	-	-	-	34 (19.1)	27 (15.2)	111 (62.4)	4 (2.2)	2 (1.1)	-	-	-	-	-	-	-	-	-
Levofloxacin	-	-	144 (80.9)	6 (3.4)	5 (2.8)	10 (5.6)	10 (5.6)	1 (0.6)	2 (1.1)	-	-	-	-	-	-	-	-	-
Gentamicin	-	-	-	36 (20.2)	16 (8.9)	65 (36.5)	56 (34.5)	-	-	-	2 (1.1)	3 (1.7)	-	-	-	-	-	-
Streptomycin	-	-	-	-	-	-	1 (0.6)	45 (25.3)	19 (10.7)	42 (23.6)	17 (9.6)	20 (11.2)	6 (3.4)	7 (3.9)	4 (2.2)	10 (5.6)	7 (3.9)	-
Sulfamethoxazole	-	-	-	-	-	-	-	64 (35.9)	5 (2.8)	2 (1.1)	16 (8.9)	20 (11.2)	5 (2.8)	7 (3.9)	-	2 (1.1)	10 (5.6)	45 (25.3)
Tetracycline	-	-	-	-	1 (0.6)	15 (8.4)	57 (32.0)	39 (21.9)	-	3 (1.7)	1 (0.6)	-	10 (5.6)	30 (16.9)	22 (12.4)	-	-	-
Trimethoprim	-	-	-	-	113 (63.5)	20 (11.2)	4 (2.2)	3 (1.7)	-	-	-	-	-	-	-	-	38 (21.3)	-

APPENDIX C

Table: Replicon sequence type of IncA/C (n=1)

Strain	Origin	Resistance gene	pMLST[a]				ST
			repA	*parA*	*parB*	*053*	
S. Reagent	dog	*qnrS*	2	2	5	1	6

[a]Plasmid multilocus sequence typing (pMLST). Allele variants for each sequenced gene (repA, parA, parB, and 053) were identified and numbered. Sequence type (ST) was assigned to the different combinations of allele variants observed in the IncA/C plasmids.

Table: Replicon sequence type of IncHI1 (n=4)

Strain	Origin	Resistance gene	pMLST[a]						ST
			1.043	1.064	1.099	1.116	1.178ac	1.259	
S. Biafra	dog	*qnrS*	1	1	1	1	0	2	16
S. Anatum		*qnrS*	1	1	1	1	0	2	16
S. Anatum		*qnrS*	1	1	1	1	0	2	16
S. Anatum		*qnrS*	1	1	1	1	0	2	16

[a]Plasmid multilocus sequence typing (pMLST). Allele variants for each locus (HCM1.043, HCM1.064, HCM1.099, HCM1.116, HCM1.178ac, and HCM1.259.) were identified and numbered. Sequence type (ST) was assigned to the different combinations of allele variants observed among the IncHI1 plasmids.

0, Null allele. When included in a profile it means that this locus is missing.

Table: Replicon sequence type of IncI1 (n=5)

Strain	Origin	Resistance gene	pMLST[a]					ST
			repI1	*ardA*	*trbA-pndC*	*sogS*	*pilL*	
S. Weltevreden	dog	-	2	1	4	1	2	101
S. Blegdam		*qnrS*	1	2	24	9	1	136
S. Malaka		-	1	2	8	6	3	60
S. Typhimurium		-	2	7	4	1	2	U
S. Anatum		*qnrS*	1	2	20	6	3	U

[a]Plasmid multilocus sequence typing (pMLST). Allele variants for each sequenced gene (*repI1, ardA, trbA-pndC*, *sogS,* and *pilL*) were identified and numbered. Sequence type (ST) was assigned to the different combinations of allele variants observed among the IncI1 plasmids.

U, unknown ST type due to no matching ST from database.

-, not detected

VITA

NAME	Thanich Chantharothaiphaichit
DATE OF BIRTH	2 Jan 1996
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Bachelor of Science in Veterinary Technology
HOME ADDRESS	151/39 Nuanchan Road, Nuanchan sub-district, Bungkum district, Bangkok, 10230