OCCURRENCE AND FATE OF VETERINARY ANTIBIOTICS AND ANTIBIOTICS RESISTANCE GENES (ARGs) FROM SWINE FARM

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A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree in Natural Resources and Environment
July 2018

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere and utmost gratitude to my supervisors Assistant Professor Charoon Sarin, Ph.D. for his continued support with advice, encouragement and guidance for making my Ph.D. thesis. Without his spiritual support and prudent guidance, I cannot complete this dissertation successfully.

Secondly, I would like to express my sincere thanks to Prof. Guang-Guo Ying, Ph.D., at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China (GIG), who has supported me for chemical, instrument and providing the facilities throughout stay in China and for giving me the opportunity to establish excellent contacts with oversea researchers and Chinese students. My thanks also go to Dr. Li-Jun Zhou and Dr. Shan Liu, who suggested me for RRLC-MS/MS instrument and interpreted. Additionally, I would also like to thank my thesis committee members Assistant Professor Puntip Klomjek, Ph.D. and Assistant Professor Kumrop Rattanasut, Ph.D. for their time and constructive advice on my research. Especially, I would like to thank Assistant Professor Kumrop Rattanasut, Ph.D. for his advice and supported me for work in the Antibiotic Resistance genes.

Special thanks to Ms. Cai Wenwen, Ms. Kanoktip Juksu and Ms. Nualjira Tachat for their friendship and their help in my laboratory work during my stay in China. I thank staffs and friends of the agricultural biotechnology laboratory, Natural resource and Environment department, Naresuan University and GIG for their kind and friendship. I would like to express my thanks to owner swine farms for their permitted me for sample collection.

The most important, I would like to thank my family for supporting me spiritually, financially and materially throughout writing this thesis and my life in general.

Finally, I gratefully acknowledge the funding sources, National Research Council of Thailand (NRCT) and the Faculty of Agriculture, Natural Resources and Environment, Naresuan University that made my Ph.D. work possible.

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Title	OCCURRENCE AND FATE OF VETERINARY ANTIBIOTICS AND ANTIBIOTIC RESISTANCE GENES (ARGS) FROM SWINE FARMS, PHITSANULOK, THAILAND
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Academic Paper	Thesis Ph.D. in Natural Resource and Environment, Naresuan University, 2017
Keywords	Occurrence, Antibiotics, Antibiotic Resistance Genes

ABSTRACT

The objective of this study were to investigate the occurrence and fate of selected veterinary antibiotics and antibiotic resistance genes in the two swine farming systems in Phitsanulok province, Thailand. The samples including feeds, water supply, flush water, effluent, sediment, feces and sludge were collected from typical and commercial swine farms. Soil samples were collected from agricultural field near the two swine farms. The liquid samples were extracted with solid phase extraction (SPE), while the solid samples were extracted with ultrasonic-assisted coupled with SPE. The target antibiotics were analyzed by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry (RRLC-MS/MS). The results showed that 7 antibiotics were found in feeds, aqueous and suspended solids of water supply at maximum concentrations of 11,695.81±16.38 μg kg^{-1} (lincomycin), 11,575.57±0.81 ng l^{-1} (ciprofloxacin) and 461,942.13±12.40 μg kg^{-1} (lincomycin), respectively. Six antibiotics were found in aqueous and suspended solids of flush water and fresh feces at maximum concentrations of 598.34±17.27 ng l^{-1} (sulfamethazine), 62,918.29±8.96 μg kg^{-1} (lincomycin) and 40,229.15±19.71 μg kg^{-1} (lincomycin), respectively. Erythromycin was found in aqueous, suspended solids and sediment of effluent at maximum concentrations of 9,614.56±1.46 ng l^{-1},

154,500.08±12.05 μg kg^{-1} and 71,123.61±23.28 μg kg^{-1}, respectively. Six antibiotics were found in dried feces, dried sludge and agricultural field soil at maximum concentrations of 26,614.38±21.47 μg kg^{-1} (lincomycin), 14,353.39±1.5 μg kg^{-1} (ciprofloxacin) and 28,909.29±2.73 μg kg^{-1} (trimethoprim). Veterinary antibiotics using in the two swine farming systems resulted in the contamination of veterinary antibiotics in waste, treated waste and utilization applying to agricultural field. Furthermore, *tet*O were found in soil samples from the two swine farms, while *tet*M was found in soil samples from commercial swine farm. Consequently, to reducing contamination of antibiotics from swine farms in the environment should be paid attention.

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ABBREVIATIONS

ASEAN	=	Association of Southeast Asian Nations
LIN	=	Lincomycin
TMP	=	Trimethoprim
SMR	=	Sulfamerazine
SM	=	Sulfameter
SMZ	=	Sulfamethazine
SCM	=	Sulfacetamide
SG	=	Sulfaguanidine
SA	=	Sulfanilamide
SDZ	=	Sulfadiazine
STZ	=	Sulfathiazole
SPD	=	Sulfapyridine
SMM	=	Sulfamonomethoxine
SCP	=	Sulfachloropyridazine
SMX	=	Sulfamethoxazole
SDM	=	Sulfadimethoxine
SDO	=	Sulfadoxine
SX	=	Sulfisoxazole
SQX	=	Sulfaquinoxaline
CFX	=	Ciprofloxacin
MAR	=	Marbofloxacin
FL	=	Fleroxacin
NFX	=	Norfloxacin
CAR	=	Carbadox
OFX	=	Ofloxacin
OMP	=	Ormetoprim
PEF	=	Pefloxacin
LFX	=	Lomefloxacin
DAN	=	Danofloxacin
EFX	=	Enrofloxacin

ABBREVIATIONS (CONT.)

SAR	=	Sarafloxacin
DIF	=	Difloxacin
ETM-H_2O	=	Anhydro erythomycin
CRM	=	Clarithromycin
LCM	=	Leucomycin
RTM	=	Roxithromycin
TAO	=	Troleandomycin
TYL	=	Tylosin
TC	=	Tetracycline
MC	=	Methacycline
NRS	=	Narasin
MNS	=	Monensin
*tet*O	=	Tetracycline (O) gene
*tet*M	=	Tetracycline (M) gene
*erm*A	=	Lincosamides (A) gene
*erm*B	=	Lincosamides (B) gene
MLSB	=	Macrolide, Lincosamides and Streptogramin B gene
*qnr*A	=	Fluoroquinolones (A) gene
*qnr*B	=	Fluoroquinolones (B) gene
BOD	=	Biological Oxygen Demand
COD	=	Chemical Oxygen Demand
TKN	=	Total Kjeldahl Nitrogen
TSS	=	Total Suspended Solid
CEC	=	Exchange Capacities
OM	=	Organic Matter
TOC	=	Total Organic Carbon
SPE	=	Solid Phase Extraction
RRLC-MS/MS	=	Resolution Liquid Chromatography-Mass spectrometry
PCR	=	Polymerase Chain Reaction
K_d	=	Adsorption-desorption distribution

ABBREVIATIONS (CONT.)

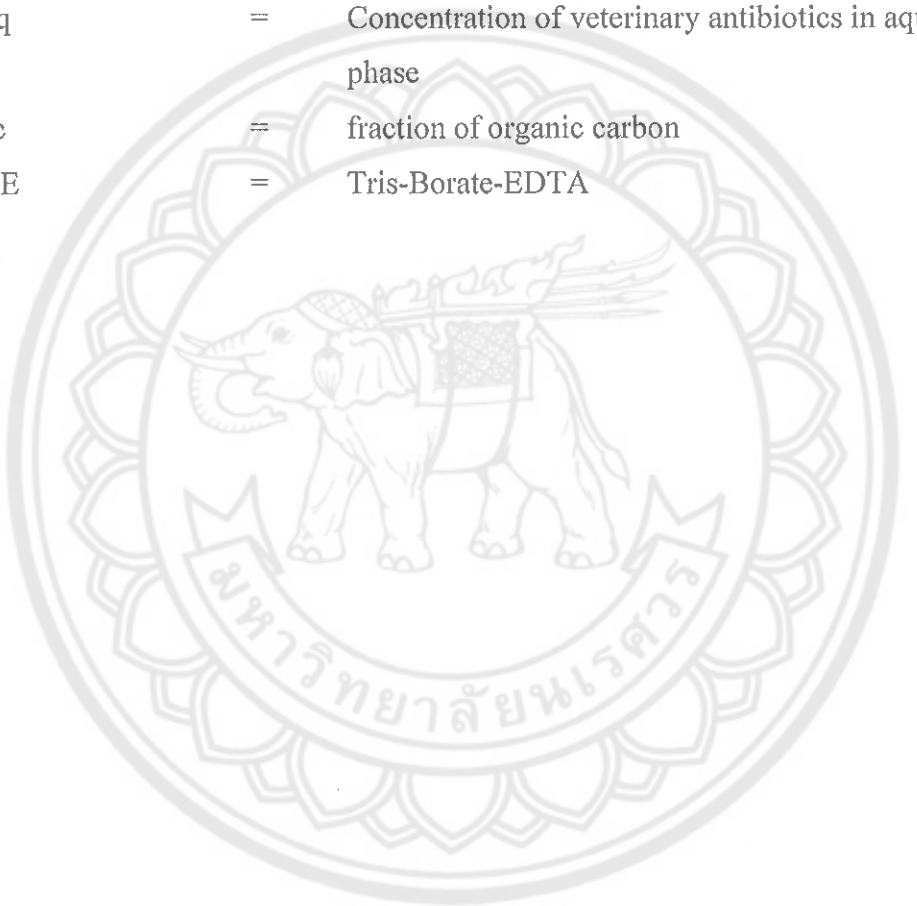
L/kg	=	Liter per kilogram
mg/L	=	Milligram per liter
log P_{ow}	=	Log octanol/water partition coefficient
K_{oc}	=	Organic carbon-water partition coefficient
pK_a	=	Acidity constant
pK_b	=	Basicity constant
Log_{Kow}	=	octanol-water partition coefficient
MW	=	molecular weight
CAS	=	Chinese Academy of Sciences
MT	=	Metric tons
mg/kg	=	Milligram per kilogram
CHN	=	China
USA	=	United States
BRA	=	Brazil
DEU	=	Germany
IND	=	India
MEX	=	Mexico
IDN	=	Indonesia
MMR	=	Myanmar
NGA	=	Nigeria
PER	=	Peru
PHL	=	Philippines
FDA	=	Food and Drug Administration
AGP	=	Antibiotic growth promoters
BMD	=	Bacitracin methylene disalicylate
ADG	=	Average daily gain
DNA	=	Deoxyribonucleic acid
RNA	=	Ribonucleic acid
MQL	=	Method Quantification Limit

ABBREVIATIONS (CONT.)

ng/L	=	Nano gram per liter
SOM	=	Soil organic matter
H^+	=	Hydrogen cations
OH	=	Hydroxide anions
AOP	=	Advanced oxidation processes
H_2O_2	=	Hydrogen peroxide
•OH	=	Hydroxyl radical
VAs	=	Veterinary antibiotics
NR	=	Not reported
Temp.	=	Temperature
NA	=	Not available
HL	=	Half-life
HGT	=	Horizontal gene transfer
EA	=	Environment Agency
Water Framework Directive	=	WFD
IS	=	Internal Standard
R.T.	=	Retention time
LC-MS/MS	=	Liquid Chromatography tandem-mass spectrometry
ARGs	=	Antibiotic Resistance genes
v/v	=	Volume/Volume
SAX	=	Strong Anion-Exchange Cartridges
HLB	=	Hydrophilic-Lipophilic Balance
CAN	=	Acetonitrile
ESI	=	Electrospray ionization source
MRM	=	Multiple-reaction monitoring
CE	=	Collision energy
QA	=	Quality assurance
QC	=	Quality control
MDL	=	Method detection limits

ABBREVIATIONS (CONT.)

MQLs	=	Quantification limits
LOD	=	Limit of detection
LOQ	=	Limit of quantitation
Cs	=	Concentration of veterinary antibiotics adsorbed by sediments or suspended solid
Caq	=	Concentration of veterinary antibiotics in aqueous phase
*f*oc	=	fraction of organic carbon
TBE	=	Tris-Borate-EDTA



CHAPTER I

INTRODUCTION

State of problem

The discovery of antibiotics has been recognized as one of the greatest advances in the history of medicine, which began the era of antibiotics. Antibiotics are compounds produced by bacteria and fungi which are capable of killing, or inhibiting, competing microbial species. They have long been considered the "miracle drugs" that would end infectious disease. Penicillin was the first true antibiotic. It discovered by Alexander Fleming, Professor of Bacteriology at St. Mary's Hospital in London in 1928. In 1940, several years before the introduction of penicillin as a therapeutic, a bacterial penicillinase was identified by two members of the penicillin discovery team (Abraham, & Chain, 1998). Since then, antibiotics have played a critical role in protecting the public's health, and are responsible for saving millions of human lives. Moreover, since 1946, the studies found that they caused animals to grow faster and put on weight more efficiently, thus leading to add antibiotics to livestock feed in industrial farms (Boyd, 2001). Today, antibiotics are routinely fed to livestock, swine, dairy, fish and cattle on industrial farms to prevent disease and promote growth in various regions of the world (Emanuele, 2010).

Antibiotics are widely used in veterinary medicine to treat and prevent health problem from infectious disease in animals. In addition, in many countries they are often added to animal feeds as antibiotic growth promoters in order to increase productivity (Page, & Gautier, 2012). During the year 1953s, The United states Food and Drug Administration (FDA) endorsed chlortetracycline and oxytetracycline as animal feed additives (Swartz, 2002) then they are widely accepted around the world. However, most antibiotics are poorly absorbed by animals (Zhu et al., 2013) and subsequently excreted with the animal wastes, resulting in as much as 30-90% of the parent compound or its metabolites being excreted in feces, urine (Sarmah et al., 2006) and ending up in manure storage tanks or lagoons (Lee et al., 2007). Antibiotics can therefore either leave the wastewater treatment plant in treated water entering rivers,

stream (Zhou et al., 2013) or become part of the sewage sludge. These compounds may be transported into the environment via surface runoff, wastewater discharge, leaching, application of manure onto agricultural fields as fertilizer (Zhou et al., 2013; Kümmerer, 2009), plant uptake (Boxall et al., 2006) and leach into groundwater, (Boxall et al., 2002; Thiele-Bruhn, 2003; Sukul, & Spiteller, 2006). In addition, swine wastewater is an important antibiotic resistance genes (ARGs) reservoir, which reflected veterinary antibiotic usage status (Sui et al., 2016). Koike et al. (2007) detected *tet*M encoding ribosomal protection protein with relative abundance of 16S rRNA in swine wastewater. McKinney et al. (2010) reported that the *sul*1 and *sul*2 encoding modified dihydropteroate synthase enzyme in resistant to sulfonamides. Jindal et al. (2006) found a high level of resistant rRNA encoded by the *erm* gene causing resistance to macrolides, lincosamides, and streptogramin B (MLSB) in swine wastewater.

Thailand is one of ASEAN country which is a major source of swine production in the world after China, EU and U.S. For Thailand, modern intensive swine production began in 1973 with the importation of breeding stock from the United Kingdom and the United States (Beeghly, 1989). Commercial development of this sector is fostered by a small number of feed mill companies which provide piglets, feeds, drugs, veterinary services and farm management expertise to contracted pig producers. Therefore, this contract system plays an important role in development of Thai commercial swine industry. In parallel with this rapid development, antibiotics are increasingly used for both treatment and growth promotion in Thailand's swine production. In addition, the typical swine farms are distributed in every region of the country. The treatment of swine disease has been not necessarily under veterinary control but the farmers have decision based on their experience and economic situation (Suriyasathaporn et al., 2012) and most of these farms lacked of the good waste management. Thus, both commercial and typical swine farms could be source of antibiotics contamination in the environment.

Therefore, this study aimed to have a screening investigation of the occurrence and fate of 41 antibiotics in in feeds, feces, flush water, effluent, sediment, sludge, water supply, agricultural soil and antibiotic resistance genes (ARGs) in agricultural soil samples from different swine farming systems in Phitsanulok

province, Thailand. The results of this study are expected to improve the understanding of the occurrence, fate and ARGs from the two swine farming systems and can be used to improve the waste management from livestock in Thailand.

Objectives of the study

1. To investigate the concentration of antibiotics in feeds, feces, flush water, effluent, sediment, sludge, water supply, agricultural soil from the two swine farming systems in Phitsanulok province, Thailand.

2. To study the fate of antibiotics from the two swine farming systems in Phitsanulok province, Thailand.

3. To investigate ARGs in agricultural soil application with swine wastewater from the two swine farming systems in Phitsanulok, Thailand.

Scope of study

This study is a survey research. Forty-one antibiotics of six classes and six ARGs were selected for this study were included;

1. The type of antibiotic

1.1 Lincosamides: lincomycin (LIN)

1.2 Diaminopyrimidines: trimethoprim (TMP)

1.3 Sulfonamides: sulfamerazine (SMR), sulfameter (SM), sulfamethazine (SMZ), sulfacetamide (SCM), sulfaguanidine (SG), sulfanilamide (SA), sulfapyridine (SPD), sulfamonomethoxine (SMM), sulfachloropyridazine (SCP), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethoxazole (SMX), sulfadimethoxine (SDM), sulfadoxine (SDO), sulfisoxazole (SX), and sulfaquinoxaline (SQX)

1.4 Fluoroquinolones: ciprofloxacin (CFX), marbofloxacin (MAR), fleroxacin (FL), norfloxacin (NFX), carbadox (CAR), ofloxacin (OFX), ormetoprim (OMP), pefloxacin (PEF), lomefloxacin (LFX), danofloxacin (DAN), enrofloxacin (EFX), sarafloxacin (SAR), and difloxacin (DIF)

1.5 Macrolides: anhydro erythomycin (ETM), clarithromycin (CRM), leucomycin (LCM), roxithromycin (RTM), troleandomycin (TAO), and tylosin (TYL)

1.6 Tetracycline: tetracycline (TC), methacycline (MC), narasin (NRS), and monensin (MNS)

2. The type of ARGs

2.1 Tetracycline resistance genes: *tet*O and *tet*M

2.2 Macrolide, Lincosamides and Streptogramin B (MLSB) resistance genes: *erm*A and *erm*B

2.3 Fluoroquinolones resistance genes: *qnr*A and *qnr*B

Scope of study areas

The study areas of this study were one typical and one commercial swine farms with different wastewater management systems in Phitsanulok province, Thailand.

Scope of sample analysis

Samples analysis methods of this study were chemical and molecular analysis.

1. Chemical analysis of wastewater characteristics, soil properties, heavy metal in soil and antibiotics concentration.

1.1 Wastewater characteristics analyzes in parameters as temperature, pH, temperature, chemical oxygen demand (COD), biological oxygen demand (BOD), total kjeldahl nitrogen (TKN), total suspended solid (TSS) with standard methods for analysis of wastewater parameters.

1.2 Soil properties analyzes in parameters as soil texture, pH, organic matter (OM), total nitrogen (TN), available phosphorus (P), available potassium (K) with standard methods for analysis of soil properties.

1.3 Heavy metal in soil analyzes as Zn, Cu, Pb, and Cd, followed the standard methods for analysis of heavy metal in soil.

1.4 Antibiotic concentrations analysis method comprised of two steps, followed Zhou et al., 2012 methods for sample preparation procedure were described in Chapter III.

1.4.1 Sample extraction and clean up with Solid Phase Extraction (SPE).

1.4.2 Antibiotic concentrations were determined by using rapid resolution liquid chromatography/tandem mass spectrometry (RRLC-MS/MS) (Zhou

et al., 2014). The target compounds were forty-one antibiotics of six classes, showed in the type of antibiotics.

2. Molecular analysis of ARGs in soil samples as *tet*O, *tet*M, *erm*A, *erm*B, *qnr*A and *qnr*B comprised of two steps;

2.1 DNA extraction by using a GenElute™ Soil DNA Isolation Kit (Sigma-Aldrich, USA). The DNA extraction steps followed the protocol provided by the manufacturer were described in Index.

2.2 DNA detection of ARGs genes by Polymerase Chain Reaction (PCR) technique. The methods for detection procedure were described in Chapter III.

CHAPTER II

LITERATURE REVIEW

This chapter described contents which relating this study included

1. Pig production in Thailand
2. The impact of swine wastewater to environment and waste management in Thailand
3. Antibiotics and veterinary antibiotics
4. Classification of commonly used veterinary antibiotics
5. Characteristics of antibiotics
6. Mechanism of action
7. Veterinary antibiotic consumption in livestock
8. Veterinary antibiotics in swine production
9. Occurrence of veterinary antibiotics in the environment
10. Fate of veterinary antibiotics in the environment
11. Antibiotic resistance in the environment
12. Mechanism of ARGs transfer in the environment
13. ARGs in soil
14. Target antibiotics and target ARGs in this study

Pig production in Thailand

In the past, more than 70% of total pig population was kept in backyard farm and pig farming mainly produced pork to meet demand inside the country (Tantasuparuk, & Kunavongkrit, 2014). In the recent decades, changes in the pig production sector have occurred in many countries, enabling increases in production of pig meat per capita and per farm (Robinson et al., 2011; Poapongsakorn, & NaRanong, 2014). The changes to the production systems included a shift from extensive, small-scale, subsistence, mixed production systems towards more intensive, large-scale, geographically-concentrated, commercially-oriented and specialized production (Robinson et al., 2011). In Thailand, modern swine breeds was first introduced in the

1960s when the first commercial pig breeds were imported from the United Kingdom by the Department of Livestock Development (DLD) and then from the United States by Kasetsart University (Robinson et al., 2011). Since then, smallholders who raise indigenous native pig breeds for both personal consumption and as a supplementary source of income have been gradually replaced by large-scale farming of improved pig breeds (Cameron, 2000). The pig revolution in Thailand corresponds to the introduction of modern technologies and farm management. The introduction of modern technology include the use of evaporated cooling housing, which provides temperatures ranging between 25 and 27 °C artificial insemination, and optimized feed ingredients and additives. These combined factors have allowed commercial farmers to raise more pigs per square meter with faster production cycles (Robinson et al., 2011). These production systems are referred to as 'intensive' in the sense that a high amount of infrastructure, technology, health care and feeds are used to increase the productivity of high-yielding animals on the farm, resulting in increased outputs (Svendsen, & Svendsen, 1997). In the pig sector, intensive production systems characterized by high input/output ratios generally, also correspond to large farm size. Consequently, in Thailand, pig production systems are classified by their farm size, expressed as number of head per farm. Less than 50 pigs being considered as smallholders (<5 pigs per holder for backyard and 5-50 pigs per holder for smallholder commercial) and holders with 50or more pigs considered as large-scale farming system (50-500 pigs per holder for small, 500-5,000 pigs per holder for moderate, and >5000 pigs per holder for large) (Ministry of Natural Resources and Environment (MONRE)).

In Thailand, pig farming systems can be categorized into three groups: i) the farrow-to-finish production system, which includes breeding pigs, producing piglets and fattening pigs in the same farm; ii) the nursery system, which only raises breeding pigs to produce piglets; and iii) the finishing system, which raises weaners until they reach market weight (Aksornphan, & Isvilanonda, 2009; Sakpuaram et al., 2002). Nowadays, two groups of pig breeds are used in Thailand: the native breeds such as Raad or Ka Done, Puang, Hailum, Kwai, and wild pigs (Rattanaronchart, 1994; Charoensook et al., 2013) and the main commercial breeds, including the Large White, Landrace, Duroc, and crosses of these (Sakpuaram et al., 2002).

The statistics of pig number in Thailand recorded from 1995-2015 showed that number of pig varied from 6-11 million pigs/year as showed in Figure 1.

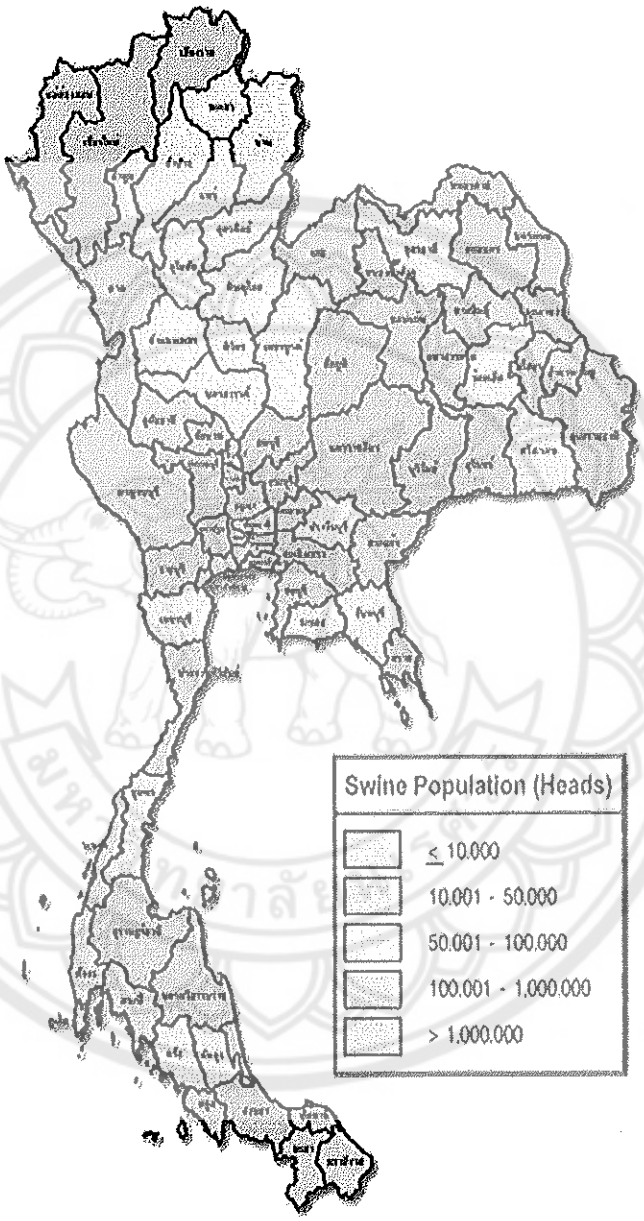


Figure 1 Pig Density in Thailand

Source: FAO, LEAD Project and OAE

The impact of swine wastewater to environment and waste management in Thailand

The Thailand Pollution Control Department (PCD) reported that the high concentration of pig farms in the central plain caused significant water pollution in rivers. The main water sources impacted by wastewater from pig farms including, Tha Chin River Shade, Chao Phraya River Shade, Bang Pakong River Shade and Songkhla Lake Shade. The impact of swine wastewater to environment such as water fouling; unusable for crop land, fishery farm and water supply, eutrophication or algae boom and lead to fouling, bad smell and also created air pollution and economic and social issues (Nintaphan, 2016). Usually, wastewater form pig farm is varied on characteristic of farming style. Characteristic of the wastewater is widely depended on farm operation (Chao, 2016). Consequently, Pollution Control Department (PCD) added pig farming to the list of regulated activities in 2001 (Poapongsakorn, & NaRanong, 2003; Tapinta et al., 2014). In order to reduce the adverse impacts of intensive pig farming, both in epidemiological and environmental terms, the Agricultural Standard Committee (Ministry of Agriculture and Cooperatives MOAC, Thailand), established the "Standard for Good Agricultural Practices for Pig Farms", which aimed to provide guidance to pig farmers and promote healthy and hygienic pig farming practices (Viriyapak et al., 2015). This document provides recommendations relating to eight topics: i) farming conditions (location, farm layout, and housing), ii) use of feed, iii) management of water, iv) overall farm management, v) animal health, vi) animal welfare, vii) the environment (in relation to proper disposal of refuse, manure, discarded carcasses, and water treatment) and viii) the keeping of records allowing tracing of animals. The standards outlined in the document are also used as guidelines for responsible agencies such as the Provincial and Regional DLD Livestock Offices to accredit and monitor pig farms (Viriyapak et al., 2008). In addition, PCD announced the standards for the discharge wastewater from the pig farms in Thailand as showed in Table 1.

Table 1 Standards for the discharge wastewater from the pig farms in Thailand

Parameters (Unit)	Maximum permitted value	
	Large farm	Medium and Small farm
pH	5.5-9.0	5.5-9.0
BOD (mg/L)	60	100
COD (mg/L)	300	400
SS (mg/L)	150	200
TKN (mg/L)	120	200

Source: Announcement from Ministry of Natural Resources and Environment (MONRE)

Antibiotics and veterinary antibiotics

Antibiotics are chemical substances, produced by micro-organisms, nowadays antibiotics are not only produced naturally by microorganisms, but they can also be synthetic or semi-synthetic. (Kümmerer, 2009), which have the ability to kill or inhibit the growth of microorganisms (Sanchez, & Demain, 2015) whereas, antimicrobial is a broad term refers to antibiotic which acts against variety of microorganisms.

According to a team of American scientists had discovered in 1950s (Ogle, 2013) that adding antibiotics to livestock feed as feed-additive increased the growth rate and cost less than general feed supplement (Ogle, 2013). The extensive use of veterinary antibiotics to treat, prevent and control disease from infectious disease in animals (Chen et al., 2012). Veterinary antibiotics are often added in animal feeds as growth promoters (Landers et al., 2012), which they play a major role in livestock production and their use has been increasing globally (Chen et al., 2012). Although veterinary antibiotics usage has become necessary because worldwide growing animal food industry (Kim et al., 2008). However, after use they are excreted and could be enter into the soil (Heise et al., 2006) through wastewater and fertilization with manure (Kümmerer, 2003) or waterways and possibly pose environmental challenges (Kumar et al., 2012).

Classification of commonly used veterinary antibiotics

1. Aminoglycosides

Streptomycin was a first antibiotic to be discovered of this class in 1943 (Mahajan, & Balachandran, 2012). The compound of usually aminoglycosides class, including three amino sugars connected with glycosidic bonds as show in Figure 2. These compounds are capable of the inhibition protein synthesis in bacteria that binding to ribosomal subunits, lastly leading to cell death (Peterson, 2008). The effectiveness of aminoglycoside activities are not only capable against gram negative bacterial and some gram positive bacterial but also these compounds must be injected because they are not absorbed during digestion. Nowadays, aminoglycosides use is limited due to issues with toxic problem (Modongo et al., 2014)

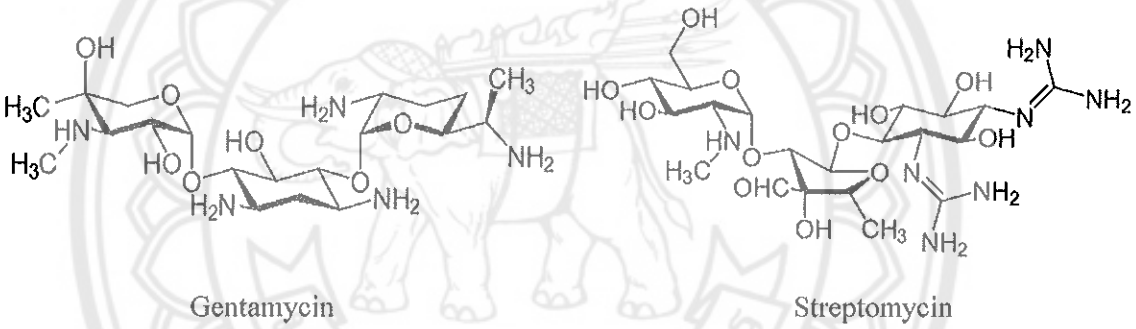
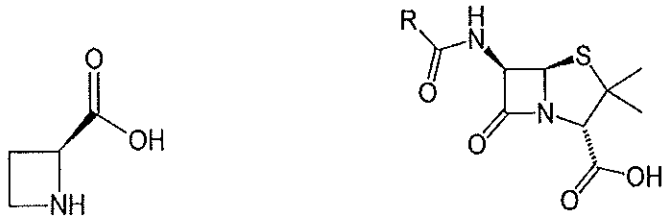


Figure 2 Structure of aminoglycosides

Source: Samanidou, & Evaggelopoulou, 2007

2. β-lactams

β-lactams are an extensive range, penicillin was the first to be discovered by Alexander Fleming. The structure of all antibiotics in this class include a four-membered cyclic amide or β-lactam (Françoise et al., 2017) as show in Figure 3. The interference proteins essential for the synthesis of peptidoglycan that is a main work of these class. Lastly, leading to cell death or inhibits their growth.



β-lactam ring Penicillin

Figure 3 Structure of a β-lactam ring

Source: Tidwell, 2008

3. Chloramphenicols

The chemical structure are compounds of ring bonded with non-ionic chlorine. It consists of two unusual components-one nitro group and a dichloroacetyl group, show in Figure 4. The molecule possesses two asymmetric carbon atoms. As a result, four optical isomers of chloramphenicol are possible. Of these isomers, only D (-) threo isomer is antibiotic ally active (Garg, 2011).

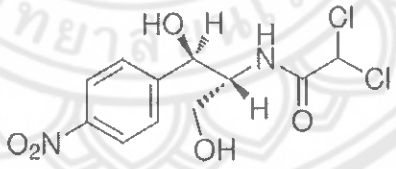


Figure 4 Structure of chloramphenicol

Source: Dasgupta, 2012

4. Diaminopyrimidines (trimethoprim)

The chemical structure are compounds of two amine groups on a pyrimidine ring which include various dihydrofolate reductase inhibitor as show in Figure 5. The activity of these compound are inhibited folic acid synthesis. (Esfahanizadeh et al., 2015).

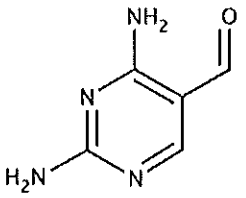


Figure 5 Structure of diaminopyrimidines

Source: Hammer et al., 2016

5. Glycopeptides

The first glycopeptide are isolated from soil which include the drug vancomycin, (Henson et al., 2015). Glycopeptides are glycosylated cyclic or polycyclic nonribosomal peptides produced from many filamentous actinomycetes group, show in Figure 6. They have been shown to inhibit gram positive bacterial cell wall synthesis by binding to the acyl-D-Ala-D-Ala peptide (Binda et al., 2014).

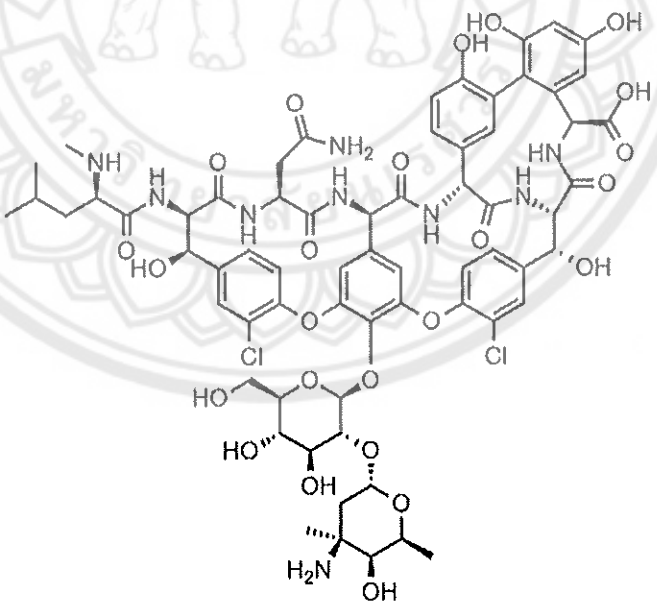


Figure 6 Structure of vancomycin

Source: Edmondson et al., 2014

6. Fluoroquinolones

The fluoroquinolones class are a large group of antibiotic synthetic antimicrobial agents that are used to treat the bacterial infections (Rubinstein, & Philippe, 2017). They contain a fluorine molecule at the 6-position of the basic quinolone nucleus (Martinez et al., 2007), show in Figure 7

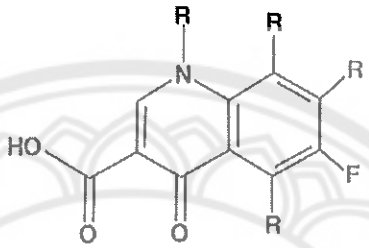


Figure 7 Structures of quinolones

Source: Maślińska, 2013

7. Lincosamides

Lincosamides from natural are produced from many Streptomyces species. For semisynthetic derivatives included clindamycin and pirlimycin. The chemical structure of lincosamides are consisted amino acid and sugar moieties, show in Figure 8. Their mode of action of lincosamides are inhibited protein synthesis (Spížek, & Řezanka, 2004)

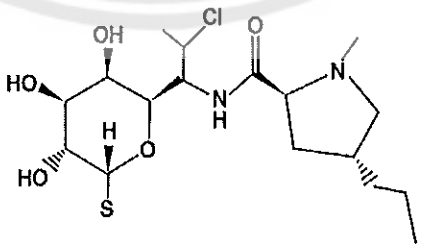


Figure 8 Structures of lincosamides

Source: Maślińska, 2013

8. Macrolides

The first, macrolides are discovered and isolated by McGuire in 1952. The chemical structure of these are include 14-, 15-, or 16-membered macrocyclic lactose rings with unusual deoxy sugars L-cladinose and D-desosamine attached (Moore, 2015), show in Figure 9.

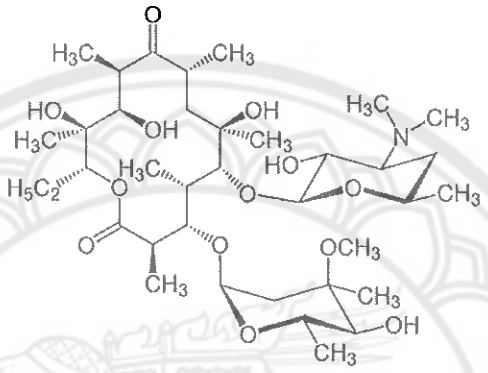


Figure 9 Structure of macrolide

Source: Seki et al., 2015

9. Tetracyclines

Tetracyclines are broad-spectrum antibiotics. Both gram-positive and negative bacterial were inhibit by these compounds. The structure of this class have four hydrocarbon rings as show in Figure 10.

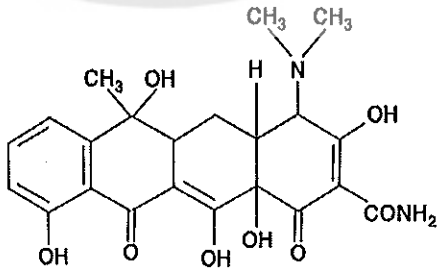


Figure 10 Structure of tetracycline

Source: Maślińska, 2013

10. Sulfonamides

Sulfonamides are the first class of antibiotic use in therapeutic and they are a major important role in medicine and veterinary practice (Smith, & Powell, 2000). The compounds which contain this functional group are called as sulfonamides, show in Figure 11. The general formula of sulfonamides RSO_2NH_2 (Lavanya, 2017). This group act by interfering with folic acid synthesis because the structure similar to para-aminobenzoic acid (PABA) of bacterial cells (Padberg, 2015).

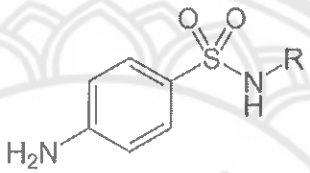


Figure 11 Structure of sulfonamide

Source: Samanidou, & Evaggelopoulou, 2007

11. Oxazolidinones

Oxazolidinones are a group of synthetic antibiotics that are containing 2-oxazolidone with a 4-substituted phenyl ring in the 3 position, show in Figure 12. They are active inhibit the gram positive bacteria and protein synthesis (Bozdogan, & Appelbaum, 2004).

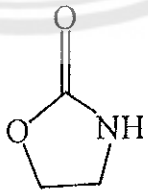


Figure 12 Structure of oxazolidinone

Source: Pandit et al., 2012

Characteristics of antibiotics

The half-life of the antibiotics in days is indicator of the degradation rate. The degradation rate is defined 10 days or less for high degradation, 10- 50 days for mediate degradation and ≥ 50 days for low degradation. For water solubility is defined >200 mg/L for high solubility, 5-200 mg/L for mediate solubility and 0-20 mg/L for low solubility. For the K_d value is the adsorption-desorption distribution coefficient (K_d). K_d value is an important parameter for considering and understanding the mobility of the antibiotics in the environment and their distribution between aqueous phase and solids phase. The K_d value is defined >200 L/kg for high sorption, 5 to 200 L/kg for mediate sorption, and 0 to 5 L/kg for low sorption (OECD, 2000). The examples of commonly used these compounds in livestock production and important physicochemical properties (Sarmah et al., 2006) as show in Table 1.

Mechanism of action

There are several major classes of antibiotics that can be classified based on their mode of antibiotic action. In general mechanism of action for antibiotics are inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, inhibition of nucleic acid synthesis, and inhibition of other metabolic activity (Serrano, 2005). The detail as show in below and figure 16 and Table 6. See table 6 for a summary of the major antibiotic classes. The selective toxicity of antibiotics lies in the differences in cellular structures between eukaryotic and prokaryotic cells. However, differences in cellular structure among bacterial species can lead to resistance to certain antibiotics.

1. **Inhibition of cell wall synthesis**

The bacterial cell wall has porous to allow transport across the cell membrane but also strong to prevent cell lysis and swelling of bacteria. Cell wall of gram negative bacterial have more complex and stronger than gram positive bacterial, resulting in gram negative bacterial cell wall are more difficult to destroy from antimicrobial agents than gram positive bacterial (Brown et al., 2015).

2. Inhibition of cell membrane function

Bacterial cell membrane is an important barriers that an inner membrane layer and extracellular flow of substances. They are composed of protein and fat. The action of this class of antibiotic can often be damage for systemic use in the mammalian host and are often poorly selective. Examples: polymixin B, amphotericin B and colistin (Thenmozhi et al., 2014).

3. Inhibition of protein synthesis

This group are interfered the bacterial protein synthesis and inhibit the ribosomes in bacterial cell by binding to either the 30S or 50S subunits of the intracellular ribosomes. Thus, they can disrupt normal cellular metabolism of bacteria, lead to cell death or growth inhibit of bacteria. Examples: aminoglycosides, macrolides, lincosamides, chloramphenicol, and tetracyclines (Tenson, & Mankin, 2006).

4. Inhibitors of nucleic acid synthesis

Groups of antibiotic are effect to nucleic acid synthesis which causes interference of the DNA or RNA replication. This drug inhibits the growth of bacteria. Examples: quinolones, fluoroquinolones, and rifampin.

5. Inhibitors of other metabolic processes

Groups of antibiotic are inhibit the metabolism of bacteria or interfere the folic acid pathway, which is an important step for produce precursors by bacteria and important for DNA synthesis. This drug in the group inhibits the growth of bacteria.

Table 2 Physicochemical properties of various veterinary antibiotics used in livestock production

Class	Type	pK_a at 25 °C	pK_b at 25 °C	Solubility (mg/l)	Vapour pressure	Henry's law constant (Pa m^3/mol)	Proton acceptors	Proton donors	$LogK_{o_w}$	MW (g/mol)
Aminoglycosides	Neomycin	12.9	9.52	-	-	$8.5x10^{-12}$ -$4.1x10^{-8}$	19	19	-3.70	614.6
	Streptomycin	-	-	-	-	-	-	-	-	581.6
	Kanamycin	7.2	-	-	-	-	-	-	-	484.5
β-lactams	Penicillins G	2.62	-	22-10,100	$1.69x10^{-18}$	$2.5x10^{-19}$-$1.2x\ 10^{-12}$	6	2	1.67	334.4
	Ampicillin	2.61	-		$1.21x10^{-19}$		-	-	1.35	349.4
	Ceftiofur	2.62	-		-		-	-	0.54	523.6
Macrolides	Tylosin	13	7.37	5,000	-	$7.8x10^{-36}$-$2.0\ x10^{-26}$	18	5	3.41	917.1
	Tilmicosin	13.16	9.81	566,000	-		15	4	5.09	869.1
	Erythromycin	8.8	-	-	-		-	-	-	733.9
	Oleandomycin	7.7	-	-	-		-	-	-	785.9
Sulfonamides	Sulfamethoxine	6.69	1.48	340	$1.05x10^{-11}$	$1.32x10^{-12}$	7	3	0.42	310.3
	Sulfamethazine	7.45	2.79	1,500	$3.64x10^{-11}$	-	6	3	0.80	278.3
	Sulfanilamide	10.6	1.9	7,500	-	$1.52x10^{-8}$	-	-	-0.62	172.2
Tetracyclines	Chlortetracycline	4.5	9.26	600	$1.57x10^{-28}$	$1.7x10^{-23}$-$4.8\ x10^{-22}$	10	7		478.9
	Oxytetracycline	4.5	9.68	1,000	$6.27x10^{-30}$		11	8		460.4
	Tetracycline	3.3-9.6	-	1,700	-		-	-		444.4

Table 2 (cont.)

Class	Type	pK_a at 25 °C	pK_b at 25 °C	Solubility (mg/l)	Vapour pressure	Henry's law constant (Pa m^3/mol)	Proton acceptors	Proton donors	$LogK_{ow}$	MW (g/mol)
Lincosamides	Lincomycin	12.9	8.78	900	$1.85x10^{-19}$	-	8	5	0.86	406.5
Fluoroquinolones	Enrofloxacin	2.74	7.11	130,000	$2.10x10^{-13}$	$5.2x10^{-17}$-$3.2x10^{-8}$	6	1	2.53	359.4
	Danofloxacin	2.73	9.13	-	$8.41x10^{-14}$		6	1	1.85	357.4
	Sarafloxacin	6.0	-	100	-		-	-	-	385.4

Source: Sarmah et al., 2006

Note: pKa = acidity constant; pKb = basicity constant; $LogK_{ow}$ = octanol-water partition coefficient; MW= molecular weight.

Table 3 Mode of action of different antibiotics generally used in animal agriculture

Mode of Action	Class/Compound				
Cell wall synthesis	**Beta-lactam**	**Glycopeptides**			
	Penicillin	Vancomycin			
	Ampicillin				
Protein synthesis	**Macrolide**	**Tetracyclines**	**Aminoglycosides**	**Chlorampenicol**	**Lincosamides**
	Tylosin	Chlortetracycline	Spectinomycin	Ceftiofur	Lincomycin
	Erythromycin	Oxytetracycline	Gentamicin		Clindamycin
Folic and nucleic	**Sulfonamides**	**Trimethoprim**	**Fluoroquinolone** (DNA replication)		
acid synthesis	Sulfamethazine	Trimethoprim	Enrofloxacin		
	Sulfameter		Ciprofloxacin		
	Sulfamerazine		Marbofloxacin		

Source: Adapted from Kumar et al., 2012

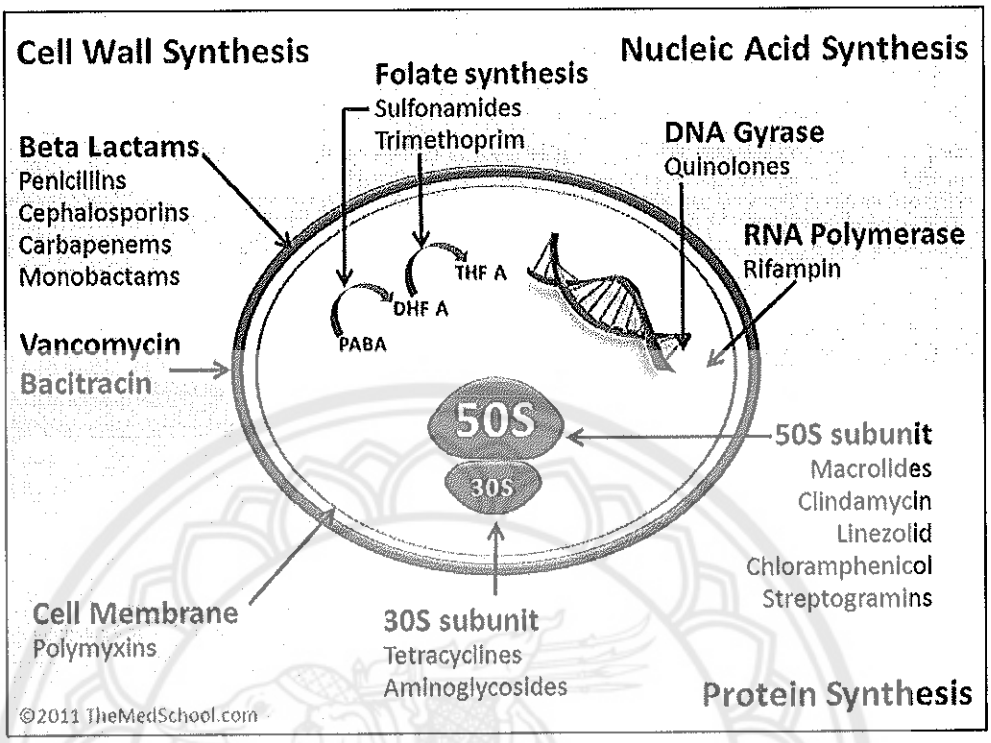


Figure 13 Antibiotics Mechanisms of action

Source: Johnson, 2011

Veterinary antibiotic consumption in livestock

Veterinary antibiotics were used in livestock adjacently parallels their discovery and usage for treatment and prevent health problem in humans. The first of antibiotic is sulfonamide that to be recommend to use in food animal in 1940s. After that in the early 50's, the newer antibiotics were discovered and available quickly led to their extensive therapeutic usage in virtually all feeds. The production and use of antibiotics increased rapidly worldwide over the last several decades since 1940s and 1950s (Hume, 2011; Fair et al., 2014). Numerous pharma- cologically active substances are used as human and animal medicines annually for treating and preventing diseases. Approximately 3,000 compounds are used as medicine (Díaz-Cruz et al., 2003) and 100,000-200,000 tons year-1 are used globally (Wise, 2002). However, the release of antibiotics into the environment has received attention in recent years (Kumar, 2012). In 2013, 73% of all antibiotics sold on earth were used in

animals, mostly as growth promoters, or as surrogates for good hygiene measures (van Boeckel, 2017). Presently, there are around 250 different chemical entities registered and currently being used as human and veterinary antibiotics (Kümmerer, 2009). The amount of all veterinary antibiotics used globally has been estimated to be 63 thousand tons per year, expected that in the next few years will continue to increase of them (van Boeckel et al. 2015).

Veterinary antibiotic consumption in the animal industry no clear information is available on the total amount of veterinary antibiotics used worldwide. Based on the amount sold in each country, the amount used is estimated, and the use may difference in each country depend on the number of livestock (Kumar et al., 2012). In the USA, antibiotics were use as feed additive is estimated around ~80% of the total amount consumption in each year. European countries (Norway, Sweden, Denmark, Austria, Switzerland, The Netherlands, and Belgium) showed a strong correlation between consumption levels for eight classes of antibiotics (Chantziaras et al., 2014) and the prevalence of antibiotic-resistant commensal *Escherichia coli* in pigs, poultry, and cattle (Kirchner et al., 2017). Since 1989, feed-additive antibiotic usage has been regulated and only non-medicated antibiotics are allowed to use in China (Zhang et al., 2015; Sarmah et al., 2006). Eight categories and a total of 56 drugs use in animal farms in China, such as Beta-Lactams, aminoglycosides, tetracyclines, and so on (Zeng et al., 2017). The scientists from Chinese Academy of Sciences (CAS) cited estimates that half the antibiotics consumed in China from the total of 150,000 to 200,000 metric tons in each year is used in livestock, especially in pig production systems (Larson, 2015). Moreover, Thailand, India and Indonesia which are examples of developing countries, there are no data at all types and amounts used of veterinary antibiotics in livestock and also lack of control the antibiotic usage as feed-additive (Sarmah et al., 2006). Whereas the Food and Agriculture Organization of the United Nations projects (FAO) estimates that the developing countries will increase in antibiotic consumption in animal products more than 70 % within 2050. Trade is also growing quickly that extended use them increases to antibiotics use and distribute antibiotic resistance globally (Elliott, 2015).

Veterinary antibiotics in swine production

Since antibiotics were first discovered, they have been widely used in swine production in many countries around the world, except European country. Veterinary antibiotics were used for swine production in several route of administration (FDA, 2014). The vast majority of veterinary antibiotics were used in feed. In swine feed, veterinary antibiotics are non-nutritive feed additives that are used for therapeutic potential at low concentrations for long time as well as capacity to growth promotion in pigs (Jacela et al., 2009; Cox et al., 2010). The use of veterinary antibiotics are also given through water at low concentration which improve the weight benefit and feed efficiency through alterations in digestion and disease protection (MacDonald, 2011). Some of the considering possible mechanisms from these compounds as growth promoter, including inhibiting the pathogens of bacteria, microbial metabolism, reduce and inhibiting the growth of bacteria, thus increase the nutrient availability for pig and increase the nutrients utilization and uptake through pig's intestinal walls (Gaskins et al., 2002). FDA approved the feed-additive antibiotic for add in swine feed, show in Table 2. Antibiotic growth promoters (AGP) have widely been used in pig diets, especially in nursery diets, to control incidences of post-weaning diarrhea and to improve growth performance (Omonijo et al., 2017).

Veterinary antibiotics can be divided into different groups or classes (Kümmerer, 2009). They have been classified earlier in several ways, subdivided according to their mechanisms of action, chemical structure (Serrano, 2005; Kümmerer, 2009) and spectrum of antibiotic activity (Calderon, & Sabundayo, 2007). Antibiotic grouping by their chemical structure and mechanism of action are commonly used in the most common classification (Botelho et al., 2015). Thus, the patterns of antibiotic activity, effectiveness, toxicity and allergic potential are show in the same or similar chemical structure (Ngan, & Writer, 2005). The major classes of veterinary antibiotic were shown below.

Table 4 Antibiotics approved for use in swine feed

Antibiotic	Indication
Bacitracin methylene disalicylate (BMD)	Increased ADG and feed efficiency Control of dysentery in growing to finishing and control of clostridial enteritis in suckling piglets
BMD + Chlortetracycline	Increased ADG and feed efficiency Treatment of bacterial enteritis and bacterial pneumonia
Bacitracin zinc, Bambermycin	Increased ADG and feed efficiency
Carbadox	Increased ADG and feed efficiency and control of swine dysentery and salmonellosis
Chlortetracycline	Increased ADG and feed efficiency, reduction of jowl abscesses, control of leptospirosis in sows and control of ileitis
Chlortetracycline + sulfathiazole + penicillin	Reduction of abscesses; treatment of bacterial enteritis; maintenance of weight gain in the presence of rhinitis
Florfenicol	Control of bacterial respiratory disease
Lincomycin	Increased ADG and feed efficiency, control of swine dysentery and ileitis and reduce severity of mycoplasmal pneumonia
Neomycin	Treatment and control of bacterial enteritis
Neomycin/oxytetracycline	Increased ADG and feed efficiency and treatment of bacterial enteritis and pneumonia and control, treatment of leptospirosis in breeders
Oxytetracycline	Increased ADG and feed efficiency
Oxytetracycline + carbadox	Treatment of bacterial enteritis and pneumonia

Table 4 (cont.)

Antibiotic	Indication
Oxytetracycline + neomycin	Prevention or treatment of bacterial enteritis and dysentery; maintenance of weight gain in the presence of atrophic rhinitis
Tiamulin	Control of dysentery and ileitis and treatment of swine dysentery
Tiamulin + Chlortetracycline	Control of dysentery; treatment of bacterial enteritis and bacterial pneumonia
Tilmicosin	Control of bacterial respiratory disease
Tylosin	Increased ADG and feed efficiency in finishers, growers and nursery pigs, control of swine dysentery, control of dysentery and ileitis
Virginiamycin	Increased ADG and feed efficiency, control of swine dysentery and treatment of swine dysentery

Note: ADG, Average daily gain

Source: Adapted from Jacela et al., 2009

Occurrence of veterinary antibiotics in the environment

The presence of antibiotics in environment is first detected three decades ago in a UK river (Watts et al., 1982). This initiated monitoring for antibiotics in the environment and studies of their environmental impact in many countries (Sarmah et al., 2006). Among the antibiotic release sources, veterinary antibiotics appear to be the most potent source as they are released into the environment through animal manure (Baguer et al., 2000), surface water, groundwater and agricultural soil has drawn the interest of researchers around the world (Kumar et al., 2012).

Veterinary antibiotics can enter the environment through application agriculture fields with manure, livestock production, wastewater, runoff, groundwater, and through leaky waste storage manure (Figure 13 and 14). The type of antibiotic

used, manure/slurry storage and waste application practices are important factors for pathways of antibiotics into the environment. USEPA, 2000 reported that 80% of antibiotics consumption in animal through livestock production and into bacteria-rich waste lagoons after that they will be spread onto the agricultural field by manure application as fertilizer. Consequently, antibiotic residues and antibiotic resistant bacteria could be easily available for transport into the environment such as agricultural soil and aquatic environment.

Amount of antibiotics are excreted with several types and dosage levels of antibiotic, animal species and age (Katz, 1980). The animal excretion of 95% could be back in active forms to the environment. Examples, chlortetracycline was used in cattle feed as growth promoter and treatment at 70 mg/head/day. It was found in fresh feces at 14 μg/g (Elmund et al., 1971). The excretion of feces and urine could be contain with veterinary antibiotics in unchanged and metabolite form and end up in the manure. So, manure usage in agriculture field as bio-fertilizer, leading to the distribution of their metabolite and parent compounds are directly exposed to the environment.

For current study on occurrence, fate, and transport of antibiotic have not only been found in wastewater, surface water and groundwater as well as in drinking water but these compounds are also reported to be detected in marine sediments too (Kümmerer, 2004).

Table 5 Concentrations of some antibiotics in swine waste

Antibiotic	Concentration	Reference
Swine feces		
Lincomycin	164 -17,000 μg/kg	Zhou et al., 2013
Trimethoprim	4.44 - 246 μg/kg	
Sulfamethazine	6.75 - 250 μg/kg	
Ciprofloxacin	9.08 μg/kg	

Table 5 (cont.)

Antibiotic	Concentration	Reference
Swine manure		
Lincomycin	ND	Zhou et al., 2012
Trimethoprim	0.00626 μg/kg	
Sulfamethazine	NR	Martínez-Carballo et al., 2007
Ciprofloxacin	ND	Zhou et al., 2012
	10,800 μg/kg	Dolliver et al., 2008
Erythomycin	43 μg/kg	Motoyama et al., 2011
	<MQL	Zhou et al., 2012
	20,000 μg/kg	Motoyama et al., 2011
Tylosin	10 mg/kg	Joy et al., 2014
Chlortetracycline	300 mg/kg	
Sugarcane/Vegetable land		
Lincomycin	ND	Zhou et al., 2013
Trimethoprim	<MQL	
Sulfamethazine	<MQL	Zhou et al., 2012
	3.69 μg/kg	Zhou et al., 2013
	NR	Wang et al., 2014
Ciprofloxacin	4.94 μg/kg	Zhou et al., 2013
	14.0 μg/kg	
Erythomycin	ND	Zhou et al., 2012
Soil near the effluent discharge		
Lincomycin	92.3 μg/kg	Zhou et al., 2013
Trimethoprim	3.20 μg/kg	
Ciprofloxacin	5.37 μg/kg	
Surface soil		
Sulfamethazine	ND-321.6 μg/kg	Li et al., 2009

Table 5 (cont.)

Antibiotic	Concentration	Reference
Wastewater (Matrix)		
Lincomycin	1420 - 166,000 ng/L (aqueous)	Zhou et al., 2013
	106 ng/L (influent)	Zhou et al., 2012
Trimethoprim	ND (effluent)	Zhou et al., 2013
	250 - 600 ng/L(aqueous)	Zhou et al., 2012
Sulfamethazine	162 ng/L (influent)	Zhou et al., 2013
	64.0 ng/L (effluent)	Zhou et al., 2012
	13.7 - 600 ng/L (aqueous)	Managaki, S et al., 2007
	19.3 ng/L (influent)	Wang, N et al., 2014
	9.3 ng/L (effluent)	Zhou et al., 2013
Erythomycin	18.5-19.2 ng/L (river water)	Zhou et al., 2012
Ciprofloxacin	89.15 ng/L (river sediment)	Zhou et al., 2013
	ND (suspended)	
	22.5 ng/L (aqueous)	
	888 ng/L (influent)	
	695 ng/L (effluent)	
	664 ng/L (digester sludge)	

Fate of veterinary antibiotic in the environment

The potential for fate of veterinary antibiotics in the environment is depends on excretion (metabolism) by livestock production, the interaction with various solid matrices in the environment and the existence of the compounds in various environment matrices (transformation). The figure of Potential pathways for veterinary antibiotics in soil and water show in figure 14 and the detail of environment processes as below.

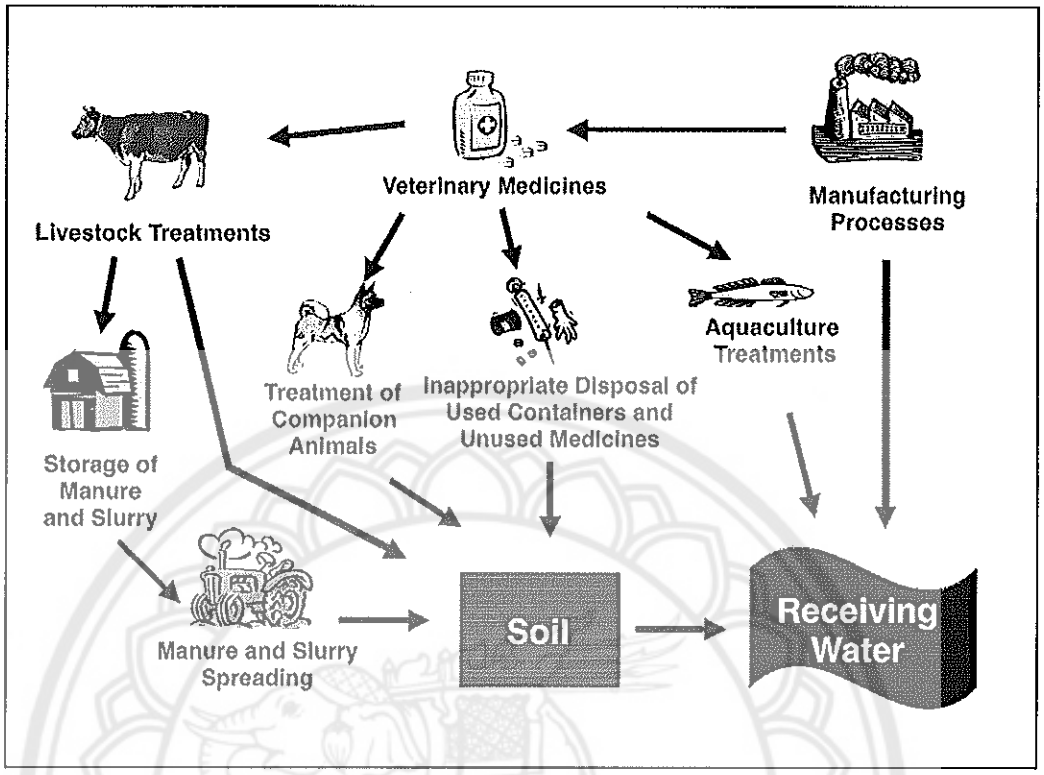


Figure 14 Potential pathways for veterinary antibiotics in soil and water

Source: Boxall et al., 2003

1. Excretion

Veterinary antibiotics are excreted by livestock production is estimated to be 75% (Sarmah, 2006) or 30-90% (Du, & Liu, 2012) of the consumed amounts that environmental exposure of antibiotic residues mainly through animal urine and faeces with metabolized and unmetabolized forms (Kaczala, & Blum, 2016). The absorption of all antibiotics after administration by animal body, the animal cannot absorbed the total amount of antibiotics, resulting they are excreted the urine and/or feces (Pikkemaat et al., 2016). The boundary of antibiotic metabolism in vivo depends on mode of application, species of animal, and age of animal (Toutain, 2010). The metabolite of antibiotics is an important factor in environmental exposure that show reduced or no antibacterial activity with compounds of them and the metabolites of some antibiotics. In addition, they could be also revert back to the parent form and active again when contaminate in the environment. Example, sulfonamides class could

be revert back to their parent compounds (Boxall, 2002; Heuer, 2008) while, they will be excreted partially as acetic acid conjugates. The contamination of antibiotics into the environment by animal excreted through a many mechanisms of the transportation that is a serious environmental threat with the emergence and development of antibiotic-resistant bacteria (Tao et al., 2010). Environment processes are responsible for antibiotics moving through livestock production into the environment by sorption, degradation and leaching processes that are an important processes between soil and water phase. However, these processes are driven with their properties such as soil texture, pH value, temperature value, organic matter value, minerals, and flow rate (Sarmah, 2006).

2. Sorption

Veterinary antibiotics relate with the sorption and desorption reactions soil solid phase (Figure 16). The sorption and desorption behavior are an important process for the fate of antibiotics in solid phase and the environment behaviors including, soil, sediment, sludge, manure and bioavailability for microorganisms (Subbiah et al. 2011).

The fates of the antibiotics solid phase in the environment is driven by the adsorption process, estimate their behaviors in the environment. However, the physicochemical properties of antibiotics effect on their adsorption behaviors with the large variations. In addition, the environment factors of solid such as the organic matter value, pH, ionic strength, metal ions also strongly impact the antibiotic adsorption processes (Wang, 2015).

The mobility antibiotics in the solid phase with the sorption process lead to distribute of antibiotics to ground water, surface water and soil. The sorption process of antibiotics is usually estimated between the soil and water distribution (Wegst-Uhrich, 2014). The important factors of parameters in soil or solid samples characteristic for adsorption are organic carbon value, clay value, soil texture, pH (OECD, 2000), ionic strength and metal ions (Wang, 2015). Moreover, the most of antibiotic sorption studies that these compounds are strongly sorbed to clay particles and soil (Table 4), whether they could still be biologically active and antibiotic resistant bacteria occur in the environment (Sarmah, 2006).

3. Degradation (transformation)

The veterinary antibiotic degradation process in environment could be found through a biotic and an abiotic processes such as biodegradation, photo-degradation and hydrolysis. These processes often play an important part in the overall distribution and elimination of veterinary antibiotics (Sarmah et al., 2006).

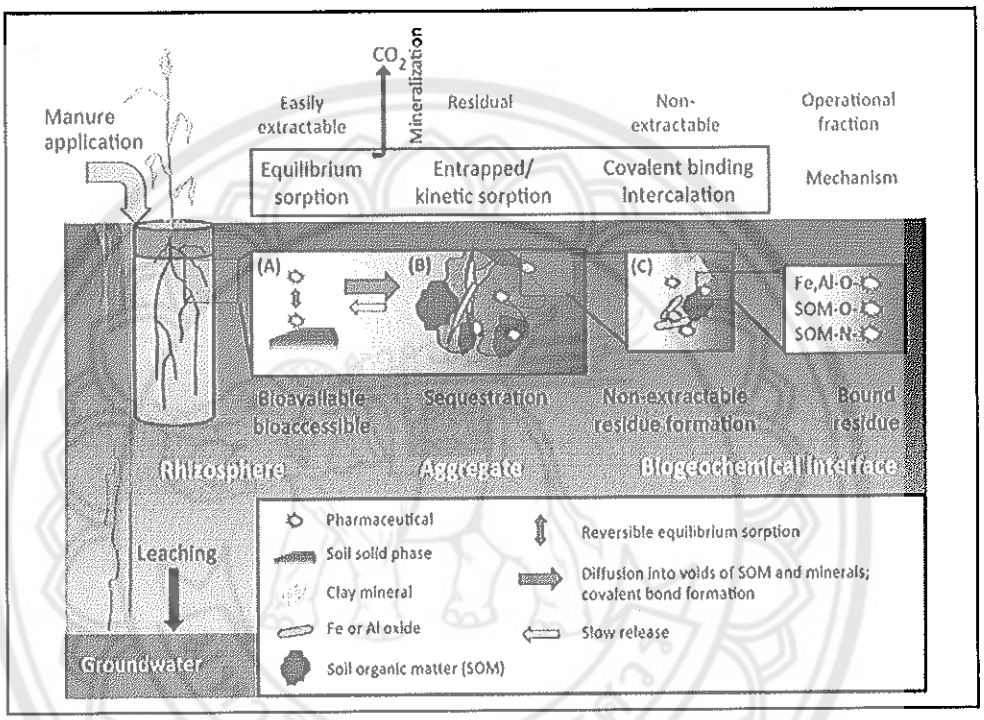


Figure 15 Fate of antibiotics in soil

Note: The bioavailable fraction (A) comprises dissolved and reversibly sorbed antibiotics. Sequestration (B) withdraws the agents from biological access and reduces their distribution as a result of kinetic sorption and diffusion into voids of soil organic matter (SOM), minerals, and microaggregates. Sequestered antibiotics can at least partly be assessed with harsh extraction methods operating at high temperatures and pressures (residual fraction). These sequestered residues can slowly be released back into bioaccessible forms. The irreversible intercalation of antibiotics in nanopores as well as the formation of covalent bonds leads to the formation of non-extractable residues (C).

Source: Jechalke et al., 2014

3.1 Photodegradation

The photodegradation process is one an important of the major transformation processes affecting the antibiotic distribution in the environment. In several reports have found that many antibiotics can be degraded with sunlight, UV, ozone, and other advanced oxidation conditions (Fernando et al., 2008). Photodegradable, water-soluble, and nonvolatile substances are particularly susceptible to photodegradation on soil surfaces (Miller, & Donaldson, 1994), and most antibiotics possess these three properties (Thiele-Bruhn, 2003). Bio-fertilizers are often distribute on soil surfaces; thus antibiotics are exposed with the ultraviolet and sunlight. However, photodegradation of antibiotics is difficult when the compounds are mixed in the turbid water of a small stream, river, soil, and sewage pipes, due to poor light penetration. The photodegradation process may differ based on the environmental conditions (Wu et al., 2010). Boreen et al., 2005 report that tetracyclines, sulfonamides and fluoroquinolones classes are eliminated in liquid phase with the photodegradation (Thiele-Brun, & Peters, 2007).

3.2 Hydrolysis

The hydrolysis process is a chemical reaction in the water which is split into hydrogen ions and hydroxide. This reaction is used to break down certain polymers, particularly those made by condensation polymerization. Such polymer degradation is usually catalyzed by either an acid, e.g., concentrated sulfuric acid, or alkali such as sodium hydroxide. A study by Paesen et al. (1995) showed that tylosin A hydrolyses into tylosin B under acidic condition, while in neutral and alkaline medium, the compound produces tylosin A-aldol on several soils (Table 10), along with number of other relatively polar decomposition products. Given the high values of pH in swine manure, understanding the hydrolysis behavior of the compound under alkaline conditions is an important (Sarmah et al., 2006). Hydrolysis and photolysis may be the major degradation processes of antibiotics in water environment (Xuan et al., 2010).

3.3 Advanced oxidation processes (AOP)

Removal of veterinary antibiotics from the environment (aqueous phase) is possible by different processes. Advanced oxidation processes (AOP) are efficient methods to degrade antibiotics. AOPs apply radicals as oxidants, which can

destroy the molecular structure of antibiotics directly. Meanwhile, ozone (O_3), hydrogen peroxide (H_2O_2) and persulfates play important roles (Guo et al., 2016). AOPs are designed to remove organic and inorganic compounds in the wastewater with oxidation process (Suzuki et al., 2016).

3.4 Biodegradation

Biodegradation of veterinary antibiotics can be achieved through various methods such as activated sludge systems, aerobic granules, bacteria, and fungi.

Biodegradation of sulfamethoxazole, sulfamonomethoxine, and sulfadimethoxine occurs within 36 h by activated sludge of wastewater treatment plant and results in reductions of 76, 81, and 70%, respectively (Yang et al., 2011). Membrane bioreactor-derived activated sludge has higher resistance and degrades 89% of TC (Prado et al., 2009). Comparing with the activated sludge, aerobic granules yield high biomass concentrations and sludge retention times that are very important of biodegradation (Shi et al., 2011). Some microorganism from aquatic environment exposed to antibiotics the white rot fungus *Phanerochaete chrysoporium* is promising for degrading SMX (Rodarte-Morales et al., 2011) could be potential develop to degrade antibiotics. Biodegradation of the common veterinary antibiotics in various environment properties show in Table 6.

Table 6 Available literature values for partitioning coefficients of selected VAs in various environmental matrices

Compound (s)	Matrices	pH	OC (%)	K_d (l kg^{-1})	K_{oc} (l kg^{-1})	References
Sulfachloropyridazine	Clay loam, sandy loam	6.5-6.8	NR	0.9-1.8		Boxall et al. (2002)
Sulfadimidine	Sand, loamy sand, sandy loam	5.2-6.9	0.9-2.3	0.9-3.5	80-170	Langhammer, & Buening (1989)
Sulfamethazine	Sand, loamy sand, sandy loam	5.2-6.9	0.9-2.3	0.6-3.2	82-208	Langhammer (1989)
Sulfapyridine	Silty loam	6.9-7.0	1.6-2.4	1.6-7.4	101-308	Thiele-Bruhn (2000)
Sulfanilamide	Whole soil, clay, sand fraction	6.7-7.0	1.6-4.4	1.5-1.7	34-106	Thiele-Bruhn et al. (2004)
Sulfadimidine	Whole soil, clay, sand fraction	6.7-7.0	1.6-4.4	2.4-2.7	61.0-150	Thiele-Bruhn et al. (2004)
Sulfadiazine	Whole soil, clay, sand fraction	6.7-7.0	1.6-4.4	1.4-2.8	37-125	Thiele-Bruhn et al. (2004)
Sulfadimethoxine	Whole soil, clay, sand fraction	6.7-7.0	1.6-4.4	2.3-4.6	89-144	Thiele-Bruhn et al. (2004)
Sulfapyridine	Whole soil, clay, sand fraction	6.7-7.0	1.6-4.4	3.1-3.5	80-218	Thiele-Bruhn et al. (2004)
Sulfathiazole	Topeka clay loam	NR	1	0.6	NR	Thurman, & Lindsey (2000)
Tylosin	Loamy sand, sand	5.6-6.3	1.1-1.6	8.3-128	553-7,990	RabØlle, & Spiild (2000)
	Silty clay, clay, sand	5.5-7.4	0.4-2.9	5.4-6690	1350-95,532	Sassman et al. (2003)
Tylosin A-aldol	Silty clay, clay, sand	5.5-7.4	0.4-2.9	516-7,740	1,290-266,896	Sassman et al. (2003)
Tylosin	Pig manure	NR	NR	45.5/270	110	Loke et al. (2002)
Tylosin	Clay loam, sandy loam	NR	2.2-4.4	66-92	NR	Gupta et al. (2003)
	Pig manure	9.0a	0.13-0.16	38.6-107.5	241-831	Kolz et al. (2005)

Table 6 (cont.)

Compound (s)	Matrices	pH	OC (%)	K_d (l kg^{-1})	K_{oc} (l kg^{-1})	References
Oxytetracycline	Loamy sand, sand	5.6-6.3	1.1-1.6	417-1,026	42,506-93,317	RabØlle, & Spiild (2000)
	Pig manure	NR	NR	83.2/77.6	195	Loke et al. (2002)
	Marine sediment	NR	NR	663, 2590	NR	Smith, & Samuelsen (1996)
Tetracycline	Clay loam	NR	1	>400	NR	Thurman, & Lindsey (2000)
Tetracycline	Clay loam, sandy loam	NR	2.2-4.4	1,147-2,370	NR	Gupta et al. (2003)
Chlortetracycline	Clay loam, sandy loam	NR		1,280-2,386		Gupta et al. (2003)
Olaquindox	Pig manure	NR	NR	20.4/9.8	50	Loke et al. (2002)
	Loamy sand, sand	5.6-6.3	1.1-1.6	0.69-1.7	46-116	RabØlle, & Spiild (2000)
Efrotomycin	Loam, silt loam, sandy loam, clay loam	5.0-7.5	1.1-4.6	8.3-290	580-11,000	Yeager, & Halley (1990)
Ciprofloxacin	Sewage sludge	6.5	37	417	1,127	Halling-SØrensen (2000)
	Loamy sand	5.3	0.7	427	61,000	Nowara et al. (1997)
Enrofloxacin	Clay, loam, loamy sand	4.9-7.5	0.73-1.63	260-5,612	16,510-99,980	Nowara et al. (1997)
Metronidazole	Loamy sand, sand	5.6-6.3	1.1-1.6	0.54-0.67	39-56	RabØlle, & Spiild (2000)
Fenbendazole	Silty loam	6.9-7.0	1.6-2.4	0.84-0.91	35-57	Thiele, & Leinweber (2000)

Note: NR = not reported; K_d = soil partition coefficient; K_{oc} = organic carbon normalized partition coefficient.

[a] pH values were after sorption experiment.

Source: Sarmah et al., 2006

Table 7 Available literature values for degradation of veterinary antibiotics in various environmental matrices

Compound (s)	Matrices	Temp. (^{O}C)	Degraded (%)	Time (days)	References
Tetracycline	Pig manure (ventilated, non-ventilated)		50	4.5-9	Kuhne et al. (2000)
	Water (ventilated, non-ventilated)		50	15-30	Kuhne et al. (2000)
	Pig manure		50-70	48	Winckler, & Grafe (2001)
Chlortetracycline	Sandy loam soil + cattle faeces	4	0	30	Gavalchin, & Katz (1994)
		20	12	30	
		30	56	30	
Oxytetracycline	Sediment slurry (aerobic)	15	50	42-46 (HL)	Ingerslev et al. (2001)
	Soil, slurry	NA	50	18-79 (HL)	Kay et al. (2004)
	Soil + cattle manure	NA	0	180	Van Gool (1993)
	Bedding + pig manure	NA	50	30 (HL)	
Tylosin	Sandy loam soil + manure	4	60	30	Gavalchin, & Katz (1994)
		20	100	30	
		30	100	30	

Table 7 (cont.)

Compound (s)	Matrices	Temp. (°C)	Degraded (%)	Time (days)	References
Tylosin	Pig manure (aerobic)	20	50	>2 (HL)	Loke et al. (2000)
Tylosin	Sand + slurry, sandy loam + slurry	NA	50	3.3-8.1 (HL)	Ingerslev, & Halling-Sørensen (2001)
Sulfonamides*	Pig manure (aerobic)	20	50	>2 (HL)	Loke et al. (2000)
Erythromycin	Sandy loam soil + cattle faeces	4	0	30	Gavalchin, & Katz (1994)
	Soil	30	100	30	Schlusener, & Bester (2006)
Ceftiofur	Soil (clay loam, sand, silty clay loam)	22	50	22-49 (HL)	Gilberstson et al. (1990)
^{14}C-Sarafloxacin	Soil (sandy loam, loam, silty loam)	22	0.5-0.6	80	Marengo et al. (1997)
Oleandomycin	Soil	20	50	23(HL)	Schlusener, & Bester (2006)
Tiamulin				26 (HL)	
Bacitracin	Sandy loam soil + cattle faeces	4	77	30	Gavalchin, & Katz (1994)
		20	67	30	
Monensin	Manure (aerobic)	NA	60-70	70	Donoho (1984)
Olaquindox	Sand + slurry, sandy loam + slurry	NA	50	5.8-8.8 (HL)	Ingerslev, & Halling-Sørensen (2001)
	Sediment slurry (aerobic)	15	50	4-8 (HL)	

Table 7 (cont.)

Compound (s)	Matrices	Temp. (^{O}C)	Degraded (%)	Time (days)	References
	Sand + slurry, sandy loam + slurry	15	50	22 (HL)	
Metronidazole	Sediment slurry (aerobic)	NA	50	13-27 (HL)	Ingerslev, & Halling-Sørensen (2001)
	Sediment slurry (anaerobic)	15	50	14-104 (HL)	
		15	50	3-75 (HL)	
Bambermycin	Sandy loam soil + cattle faeces	4	0	30	Gavalchin, & Katz (1994)
		20	100	30	
		30	100	30	
Virginiamycin	Silty sand	25	50	87-173 (HL)	Weerasinghe, & Towner (1997)

Note: HL = half-life; a = first spike, b = second spike; NA = not available.

* Sulfacetamide, sulfabenzamide, sulfamethoxypyridazine, carbutamide, sulfamerazine, sulfameter, sulfadoxine, sulfanilamide, sulfadimidine, sulfadiazine, sulfadimethoxine, sulfapyridine, sulfachloropyridazine.

Source: Sarmah et al., 2006

Antibiotic resistance in the environment

Antibiotics have long been considered the "miracle drug" that can be used to kill or inhibit bacterial growth and are used to treat bacterial infections in both humans and animals. However, bacteria can be adapted by themselves to survive in natural environment as the consequence of mutation (Martínez, & Baquero, 2000) or inherent resistance and they can be adapted under antibiotic conditions with the horizontal gene transfer (Davies, 1994) as acquired resistance, even as the scientist develop new antibiotics. In recent years, much attention has been given to the increase in antibiotic resistance. As more microbial species and strains become resistant, antibiotic resistant bacteria are now found in large numbers in virtually every ecosystem on earth.

In recent years, antibiotic resistant bacteria have been isolated from virtually every environment on earth, even the areas have never used antibiotics before. Moreover, resistance genes can be spread far wider than once believed and a pool of resistance is developing in non-pathogenic organisms found in humans, animals, and the environment. These non-pathogenic organisms serve as a source from which pathogens can acquire genes conferring resistance, and in turn, they can become resistant by acquiring genes from pathogens discharged into the environment via sewage or agricultural runoff. Thus, dissemination of resistant bacteria is not only a problem of the resistant pathogens themselves, but also availability of resistance genes to pathogens via gene transfer.

Mechanism of ARGs transfer in the environment

Resistance in bacterial populations in the environment is not new, but understanding the potential for the development of resistant bacteria from the use of antibiotics as growth promoters is beginning to be examined (Hirsch et al., 1999; Kümmerer, 2009). The development of resistance in bacteria occurs primarily through two mechanisms, "inherited" and secondary resistance. Inherited resistance occurs through bacterial cell division, while secondary resistance involves the transfer of plasmids between microorganisms. The origin of the genes is link between ARGs in human pathogens and those found in commensal microorganisms, with several common bacteria resistance taxa such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* coming from the natural environment (Wright,

2010). Generally, bacterial resistance to antibiotics can be acquired by horizontal gene transfer (HGT) or by spontaneous mutation in target gene (Hassan et al, 2012). In fact, ARGs could be associated with a transposable element. The mobility of ARGs involves the transference of genetic material to other bacteria of the same or different species (Thomas, & Nielsen, 2005). Antibiotic resistance is everywhere, and consequently efforts are being devoted to understanding the origin of resistance genes, particularly among the vast majority of not-yet-culturable environmental bacteria. For instance, the close association between people, animals, and the environment can be responsible for the evolution and spread of antibiotic resistance (Gautam, & Morten, 2014).

Genetic mechanisms involved in lateral exchange of ARGs are driven by the three main mechanism: 1) Conjugative transfer (e.g., via plasmids, transposons, and integrons), 2) Transduction by bacteriophage and 3) Transformation which is dependent on the native competent state of bacteria as well as cells acquiring induced competency. More recently, novel phage-like gene transfer agents (GTA) have been reported in diverse environmental isolates (Stanton, 2007), suggesting additional mechanisms of gene transfer that may also be significant in soil systems (Chee-Sanford et al., 2009). Thus, these mechanisms of horizontal gene transfer focuses on the mechanisms of gene transfer in the context of soil environmental conditions in this literature.

1. Conjugation

Conjugation is the transfer of DNA through a multi-step process requiring cell to cell contact via cell surface pili or adhesins. It is facilitated by the conjugative machinery which is encoded either by genes on autonomously replicating plasmids or by integrative conjugative elements in the chromosome (Smillie et al., 2010; Wozniak, & Waldor, 2010). Additionally, this conjugative machinery may enable the mobilization of plasmids that are non-conjugative, as observed for e.g., the exceptionally broad host range IncQ plasmids (Meyer, 2009). Because conjugation is dependent on direct cell contact, cell densities and the environment in which the bacteria reside plays a large role in the outcome frequency of conjugation events. In general, it is thought that conjugative mechanisms of gene transfer in the environment are important in the spread of genetic information, occurring over a broad host range of genera and species, and explains incidences of similar DNA sequences found

among distantly related bacterial species. It is facilitated by the conjugative machinery which is encoded either by genes on autonomously replicating plasmids or by integrative conjugative elements in the chromosome (Smillie et al., 2010; Wozniak, & Waldor, 2010). Triparental mobilization of DNA can occur when a conjugative plasmid is transferred from a parent cell to a recipient containing a nonconjugative plasmid, and both plasmids may be subsequently transferred to a recipient containing neither plasmid. While such triparental matings occur at lower frequencies than biparental matings, such a mechanism of DNA transfer has been shown to occur in soil bacteria (Trevors, 1999; Lesická-Hupková et al., 1996).

Many ARGs are harbored on mobile genetic elements (MGEs) such as transposons, integrons, or plasmids and can be readily transferred between members of the same species, and between bacteria. Several microcosm studies have documented plasmid transfer in soil environments, and plasmid transfer from introduced bacteria to soil native bacteria (Andrews et al., 2004; Heuer et al., 2002; Lee, & Stotzky, 1999; DiGiovanni et al., 1996; Wellington et al., 1992).

2. Transformation

Transformation is a way in which MGEs move around to different positions within the genome of a single cell. Transposons are sequences of DNA, also called jumping genes or transposable genetic elements that move directly from one position to another within the genome. During transformation, the insertion of sequences can both cause mutation and change the amount of DNA in the genome. Bacteria multiply by binary fission. The rate of bacterial growth is dependent upon the specific organism; *Escherichia coli* in nutrient broth will replicate in 20 minutes, whereas Mycobacterium tuberculosis has a doubling time of 28 to 34 hours. Initiation of replication begins at a unique genetic site, referred to as the origin of replication. Chain elongation occurs in a bidirectional mode. The addition of nucleotides occurs in the 5′ to 3′ direction; one strand is rapidly copied (the leading strand) while the other (the lagging strand) is discontinuously copied as small fragments that are enzymatically linked by way of ligases and DNA polymerases. As the circular chromosome unwinds, topoisomerases, or DNA gyrases, function to relax the supercoiling that occurs. Finally, termination and segregation of newly replicated

genetic material takes place, linked to cellular division, so that each daughter cell obtains a full complement of genetic material (Actor, 2012).

3. Transduction

Bacteriophages play an important role in shaping the bacterial microbiome in any environment. Through specialized or generalized transduction, bacteriophages can transfer genes that are advantageous to their microbial hosts, in turn promoting their own survival and dissemination (Modi et al., 2013). The transferable DNA sequences range from chromosomal DNA to MGEs (plasmids, transposons and genomic islands) (Brown-Jaque et al., 2015). The mobilization or transfer of ARGs by bacteriophages has been documented for various bacterial species: the transduction of erythromycin (Hyder, & Streitfeld, 1978), tetracycline or multiple resistances between strains of *Streptococcus pyogenes* (Ubukata et al., 1975); the transfer of tetracycline and gentamicin resistance between enterococci (Mazaheri Nezhad Fard et al., 2011); the carriage of β-lactamase genes by bacteriophages in *Escherichia coli* (Billard-Pomares et al., 2014) and Salmonella (Schmieger , & Schicklmaier, 1999); or the transfer of antibiotic resistance plasmids in MRSA (Varga et al., 2012).

Several studies have used qPCR to detect ARGs in bacteriophages from wastewater samples (Colomer-Lluch et al., 2014a, 2014b), wastewater and sludge derived from wastewater treatment plants (Calero-Caceres et al., 2014), and hospital and wastewater treatment plant effluents (Marti et al., 2014), indicating that bacteriophages are significant reservoirs of ARGs).

Considering certain bacteriophages have been reported to have a wide host range that crosses between different species (Mazaheri Nezhad Fard et al., 2011) or even different taxonomic classes (Jensen et al., 1998), the observation of the plethora of ARGs carried by bacteriophages in various bacterial communities and environments provides renewed insights into the role of transduction in the dissemination of ARGs in microbial ecosystems.

Antibiotics Resistance in soil

Soil samples, which are characterized as a complex and dynamic environmental system, comprising higher microbial diversity of bacteria, archaea, fungi, viruses, and protozoa (Young, & Crawford, 2004), when compared to other natural environments

such as freshwater or extreme habitats (Sleator et al., 2008). The ecology and activity of soil microbial communities depend on biotic or/and abiotic factors such as soil pH, nutrient availability, water availability, and vegetation cover above ground (Fierer et al., 2007). Various microorganisms inhabit the water, soil and sediment environments, but variability exists due to physiochemical properties, pH, moisture, nutrients, light, and temperature present in each niche (Kümmerer, 2009). Bacteria are important to invertebrates as nutrients and symbionts within their gut (Wetzel, 2001).

Recently, the detection of antibiotics have occurred in the environment and natural soils (Kümmerer, 2013) and the entry of these compounds could affect the population dynamics of microorganism and alter their ecological functions in water, sediment and soil. The additional influx of antibiotics into the environment has raised concerns that they could cause antibiotic resistant strains of bacterial populations to increase, leading to adverse effects in ecosystem. For the agricultural part, animal manure is an important reservoir of antibiotic-resistant bacteria, antibiotic-resistance genes (collectively known as the "resistome"), and pathogens (Zhou et al., 2012; Zhu et al., 2013). Although antibiotic use increases antibiotic-resistance genes and resistant bacteria in manure (Looft et al., 2012), antibiotic-resistant bacteria are also abundant in manure from animals with no history of antibiotic treatment, indicating the natural presence of bacteria intrinsically resistant to antibiotics in animal gastrointestinal tracts (Stanton et al., 2011). Antibiotics are a natural mechanism used by microbes in their natural ecology for millions of years, the abundance of natural antibiotics seem to be low on average and seems to be restricted to the nearest surroundings, i.e. the microenvironment of the bacteria (Kümmerer, 2013). Antibiotics have been detected in soil in concentrations in the mg kg-1 range (Kümmerer, 2013) that they may influence cell function and genetic expression of antibiotic resistance (Salyers, 2002). Antibiotic-resistant bacteria and antibiotic-resistant genes found in soils where manure has been added by animals or by spreading (Avant, 2016), tylosin disappeared soon after the application of manure (Kümmerer, 2013). Three potential streams of concern related to antibiotic use in agriculture and specifically manure management including, 1) animals excrete antibiotics or their metabolites that may favor selection of antibiotic resistant organisms in the soil or receiving environment, 2) manure may contain potentially pathogenic organisms that are resistant to antibiotics, and 3) manure may

contain antibiotic resistant genes, which may be transferred to other organisms in the receiving environment.

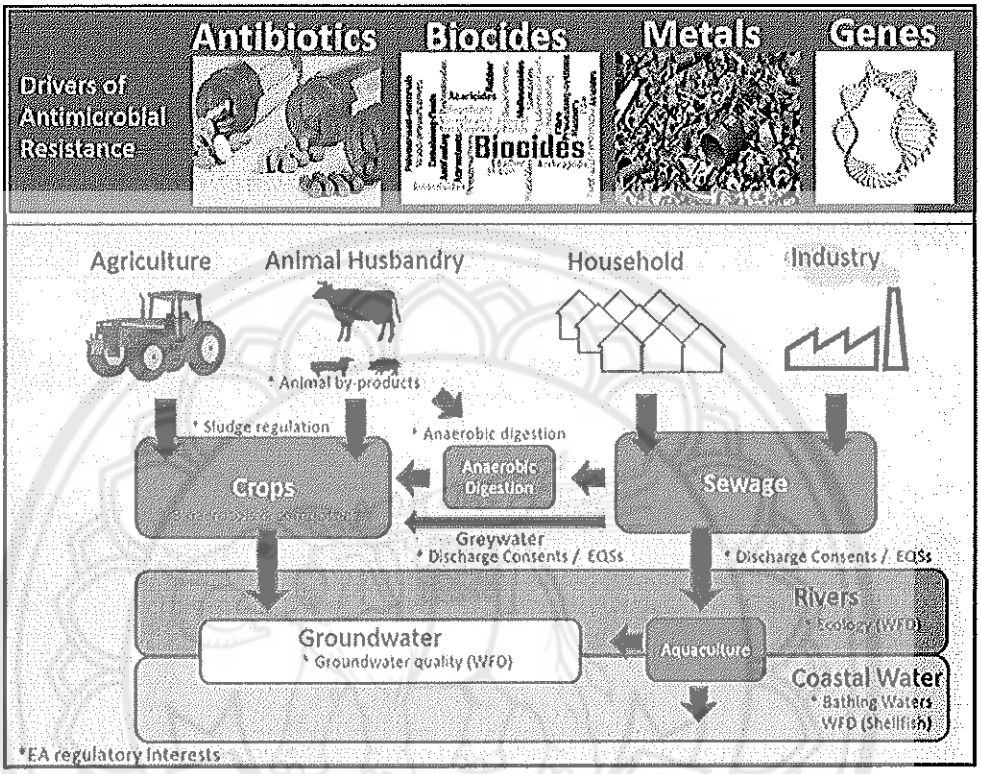


Figure 16 Schematic of the hot-spots and drivers of antimicrobial resistance

Note: The environmental compartments that are currently monitored or regulated by the Environment Agency (EA; England) are denoted by an asterisk in red. WFD, Water Framework Directive.

Source: Singer et al., 2016

Target antibiotics and target ARGs in this study

1. Target antibiotics

The antibiotic compounds in this study include forty-one antibiotics belonging to eight groups, including

1.1 Sulfonamides class: sulfamerazine (SMR), sulfameter (SM), sulfamethazine (SMZ), sulfacetamide (SCM), sulfaguanidine (SG), sulfanilamide (SA), sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SPD), sulfamonomethoxine (SMM),

sulfachloropyridazine (SCP), sulfamethoxazole (SMX), sulfadimethoxine (SDM), sulfadoxine (SDO), sulfisoxazole (SX), and sulfaquinoxaline (SQX)

1.2 Tetracyclines class: tetracycline (TC) and methacycline (MC)

1.3 Lincosamides class: lincomycin (LIN)

1.4 Macrolides class: erythromycin (ETM), clarithromycin (CRM), leucomycin (LCM), roxithromycin (RTM), Oleandomycin (ODM), tylosin (TYL)

1.5 Fluoroqinolones class: ciprofloxacin (CFX), marbofloxacin (MAR), fleroxacin (FL), norfloxacin (NFX), carbadox (CAR), ofloxacin (OFX), pefloxacin (PEF), lomefloxacin (LFX), danofloxacin (DAN), enrofloxacin (EFX), sarafloxacin (SAR), and difloxacin (DIF)

1.6 Diaminopyrimidines: trimethoprim (TMP), ormetoprim (OMP)

1.7 Ionophores class: narasin (NRS)

1.8 Other class: and monensin (MNS).

These compounds were selected as they have different classes as widely used for human and swine production. Selection was also based on the detection in wastewater reported by other studies. The chemical structure and physicochemical properties of the antibiotic are shown in Table 8.

Table 8 Antibiotic chemical structure and selected physical-chemical properties

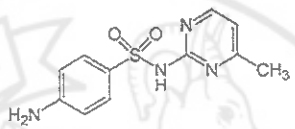
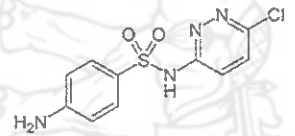
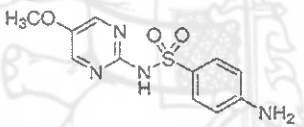
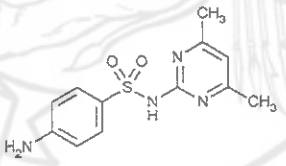
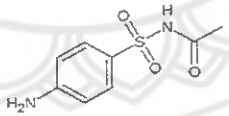
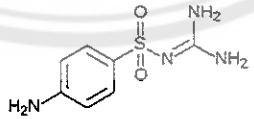
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow} [a]	pKa
Sulfonamides							
1	Sulfamerazine	264.31	$C_{11}H_{12}N_4O_2S$		202	0.14	6.99
2	Sulfachlorpyridazine	284.72	$C_{10}H_9C_1N_4O_2S$		7000	0.31	5.90
3	Sulfameter	280.30	$C_{11}H_{12}N_4O_3S$		730	0.41	6.80
4	Sulfamethazine	278.33	$C_{12}H_{14}N_4O_2S$		1,500	0.80	7.59
5	Sulfacetamide	214.24	$C_8H_{10}N_2O_3S$		12,500	-0.96	4.30
6	Sulfaguanidine	214.24	$C_7H_{10}N_4O_2S$		2,200	-1.22	11.25

Table 8 (cont.)

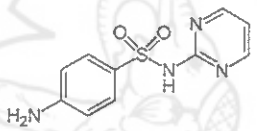
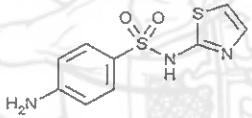
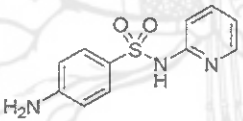
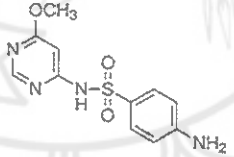
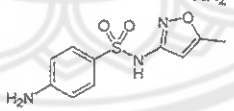
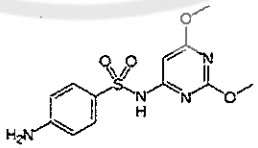
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow} [a]	pKa
7	Sulfanilamide	172.20	$C_6H_8N_2O_2S$		7,500	-0.62	10.58
8	Sulfadiazine	250.28	$C_{10}H_{10}N_4O_2S$		77	-0.09	6.36
9	Sulfathiazole	255.32	$C_9H_9N_3O_2S_2$		373	0.05	7.2
10	Sulfapyridine	249.29	$C1_1H_{11}N_3O_2S$		270	0.35	8.43
11	Sulfamonomethoxine	280.30	$C_{11}H_{12}N_4O_3S$		4,030	0.70	6.33
12	Sulfamethoxazole	253.28	$C_{10}H_{11}N_3O_3S$		610	0.89	5.98
13	Sulfadimethoxine	310.33	$C_{12}H_{14}N_4O_4S$		343	1.63	6.91

Table 8 (cont.)

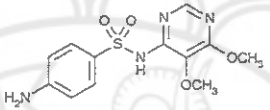
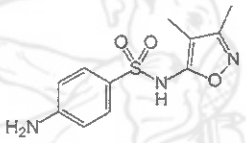
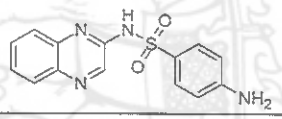
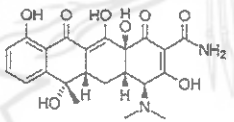
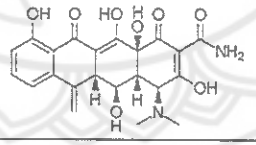
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow} [a]	pKa
14	Sulfadoxine	310.33	$C_{12}H_{14}N_4O_4S$		2,700	0.7	6.12
15	Sulfisoxazole	267.3	$C_{11}H_{13}N_3O_3S$		300	1.01	5.00
16	Sulfaquinoxaline	300.37	$C_{14}H_{12}N_4O_2S$		7.5	1.68	6.79
Tetracyclines							
17	Tetracycline	444.44	$C_{22}H_{24}N_2O_8$		1,700	-1.19	3.30
18	Methacycline	442.42	$C_{22}H_{22}N_2O_8$		7,550	-1.37	2.88

Table 8 (cont.)

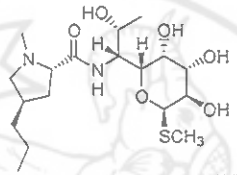
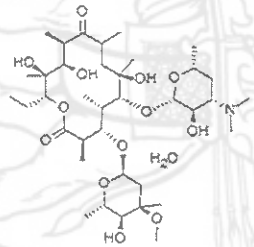
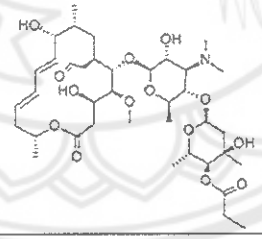
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow}[a]	pKa
Lincosamides							
19	Lincomycin	406.54	$C_{18}H_{34}N_2O_6S$		927	0.56	3.24
Macrolides							
20	Erythromycin-H_2O	733.94	$C_{37}H_{65}NO_{12}$		1.44	3.06	8.88
21	Leucomycin	686.81	$C_{39}H_{65}NO_{14}$				

Table 8 (cont.)

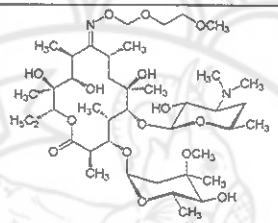
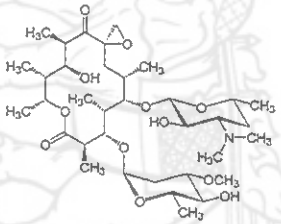
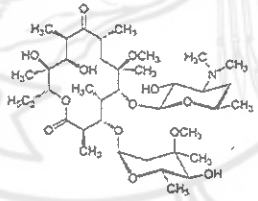
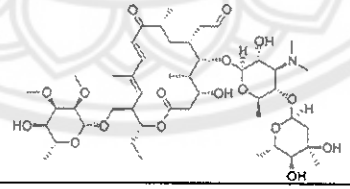
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow}[a]	pKa
22	Roxithromycin	837.05	$C_{41}H_{76}N_2O_{15}$			2.75	9.17
23	Oleandomycin	687.86	$C_{35}H_{61}NO_{12}$		15.5	1.69	8.84
24	Clarithromycin	747.95	$C_{38}H_{69}NO_{13}$		0.342	3.16	8.99
25	Tylosin	916.1	$C_{46}H_{77}NO_{17}$		5	1.63	7.73

Table 8 (cont.)

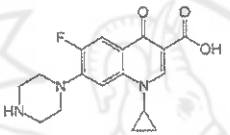
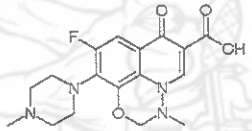
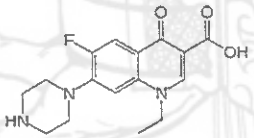
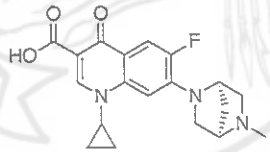
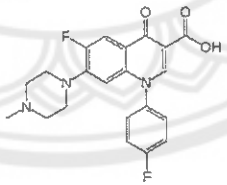
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow} [a]	pKa
Fluoroqinolones							
26	Ciprofloxacin	331.35	$C_{17}H_{18}FN_3O_3$		30,000	0.4	6.09
27	Marbofloxacin	370.41	$C_{17}H_{19}FN_4O_4$				6.02
28	Norfloxacin	319.34	$C_{16}H_{18}FN_3O_3$		17,800	-1.03	5.77
29	Danofloxacin	357.37	$C_{19}H_{20}FN_3O_3$			1.85	6.43
30	Difloxacin	399.39	$C_{21}H_{19}F_2N_3O_3$		1,330	0.89	5.85

Table 8 (cont.)

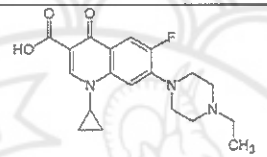
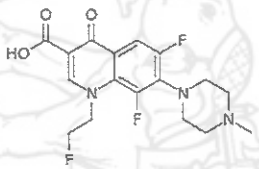
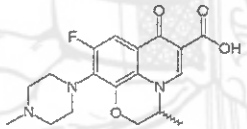
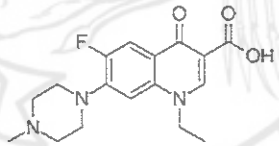
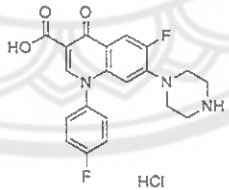
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow}[a]	pKa
31	Enrofloxacin	359.40	$C_{19}H_{22}FN_3O_3$		130,000	1.1	5.88
32	Fleroxacin	369.34	$C_{17}H_{18}F_3N3O_3$		7,320	0.24	5.44
33	Ofloxacin	361.37	$C_{18}H_{20}FN_3O_4$		2,830	0.36	5.45
34	Pefloxacin	333.36	$C_{17}H_{20}FN_3O_3$		1,140	0.27	5.66
35	Sarafloxacin	385.4	$C_{20}H_{17}F_2N_3O_3$		100	1.07	5.74

Table 8 (cont.)

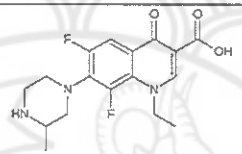
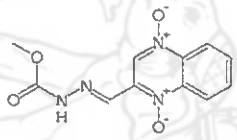
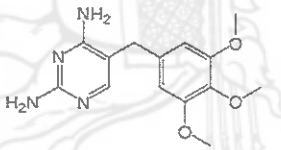
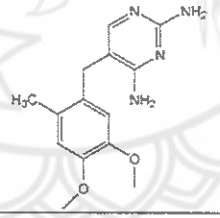
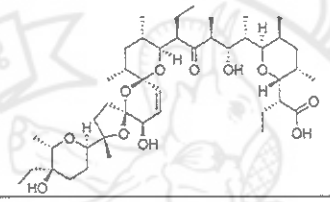
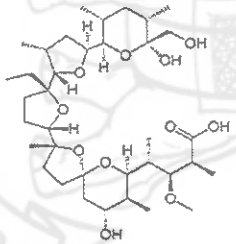
No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow} [a]	pKa
36	Lomefloxacin	351.35	$C_{17}H_{19}F_2N_3O_3$		27,200	-0.3	5.64
37	Carbadox	262.22	$C_{11}H_{10}N_4O_4$		15,000	-1.37	4.30
Diaminopyrimidines							
38	Trimethoprim	290.32	$C_{14}H_{18}N_4O_3$		400	0.91	7.12
39	Ormetoprim	274.32	$C_{14}H_{18}N_4O_2$		1,540	1.23	

Table 8 (cont.)

No.	Class/Antibiotics	M.W.	Formula	Molecular structure	Solubility (mg/L)	Log K_{ow}[a]	pKa
Ionophores							
40	Narasin	765.04	$C_{43}H_{72}O_{11}$		102-681	4.9-6.2	7.90
Other class							
41	Monensin	670.88	$C_{36}H_{62}O_{11}$		3.00E-03	5.430	6.65

Note: IS = Internal Standard; M.W. = molecular weight; R.T. = retention time; a Kow: the octanol-water partition coefficient; pKa: acidity constant

Source: U.S. National Library of Medicine ChemIDPlus Advanced. (http://chem.sis. nlm.nih.gov/chemidplus/), 28 September, 2017

2. Target of ARGs

2.1 Tetracycline resistance genes: *tet*O and *tet*M

The translational apparatus represents one of the major targets within the bacterial cell for antibiotic treatment (Wilson, 2009). Tetracyclines are broad-spectrum antibiotic agents that bind to elongating ribosomes and inhibit delivery of the ternary complex EF-Tu, GTP and aminoacylated-tRNA (EF-Tu•GTP•aa-tRNA) to the A-site (Wilson, 2009). Consistently, crystal structures of the small (30S) ribosomal subunit in complex with tetracycline reveal the primary binding site to be located in helix 34 (h34) of the 16S rRNA, in a position overlapping with the anticodon stem-loop of A-site tRNA (A-tRNA) (Brodersen, 2000; Pioletti, 2001). The widespread use of tetracyclines during the past 60 years has led to an increase in acquired tetracycline resistance determinants among clinically important pathogenic bacteria, limiting the utility of many members of this class (Roberts, 2005). Of the variety of tetracycline-specific resistance mechanisms, efflux and ribosome-protection are the most common. The third generation of tetracycline derivatives, such as tigecycline (Tgc), display enhanced antimicrobial activity, overcoming efflux and ribosome protection mechanisms (Chopra, 2002; Grossman et al., 2012).

2.2 Macrolide, Lincosamides and Streptogramin B (MLSB) resistance genes: *erm*A and *erm*B

Erythromycin resistance genes are widely disseminated among many species of bacteria; over a dozen resistance determinants have been described (Weisblum, 1995). In *Staphylococcus aureus*, erythromycin resistance is usually due either to ribosomal modification by 23S rRNA methylases mediated primarily by *erm*A, ermB, or ermC or to active efflux of the antimicrobial agent by an ATP-dependent pump mediated by msrA. *erm*A is most often harbored on the transposon Tn554, which also encodes spectinomycin resistance, while *erm*B is often associated with transposon Tn551 and the penicillinase plasmid, pI258 (Mitsuhashi, 1963; Novick et al., 1979). All of the erm determinants confer cross-resistance to macrolides, lincosamides, and streptogramin B agents (MLSBphenotype) (Hays et al., 2014).

2.3 Fluoroquinolones resistance genes: *qnr*A and *qnr*B

Since the first plasmid-mediated quinolone resistance (PMQR) was reported in 1998 for a *Klebsiella pneumoniae* isolate from the United States (Martínez

et al., 1998), three PMQR mechanisms have been discovered. The first PMQR mechanism involves *qnr* genes that have been reported worldwide in various enterobacterial species (Wang et al., 2003; Wang et al., 2004). The second consists of the AAC(6′)-Ib-cr gene, which encodes a new variant of the common aminoglycoside acetyltransferase that is capable of acetylating the piperazinyl substituent of some fluoroquinolones (Robicsek et al., 2006) and thereby reducing their activities. A novel plasmid-mediated fluoroquinolone efflux pump protein, *Qep*A, has recently been reported simultaneously from Japan (Yamane et al., 2007) and Europe (Périchon et al., 2006) as the third PMQR mechanism. A strong association of quinolone resistance with the production of extended-spectrum β-lactamases (ESBLs) or plasmid-mediated *Amp*C β-lactamases (pACBLs) has been observed (Jacoby et al., 2006; Li et al., 2005; Wang et al., 2003). The association between *qnr*A and ESBL determinants for SHV-5 (Nazic et al., 2005; Wang et al., 2003), SHV-7, CTX-M-9, CTX-M-14 , CTX-M-15 (Jacoby et al., 2006), and VEB-1 or pACBL determinants for DHA-1 and FOX-5 has been reported repeatedly. Similarly, *qnr*B has been reported to be located on plasmids carrying bla genes for CTX-M-15, SHV-12 (Jacoby et al., 2006), or SHV-30 (Gay et al., 2006) ESBLs.

CHAPTER III

METHOD

This chapter described the materials and methods of this study, the details of each part is described below.

Overall process of the study

This study, the samples were collected from typical swine farm and commercial swine farms with different farming systems in July, 2016 as water supply in swine farm, flush water, effluent, swine feed, feces, sediment from an oxidation pond and lagoon sediment, sludge and drained agricultural soil receiving swine wastewater. All samples were quantified by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry (RRLC-MS/MS) and were analyze environment parameter and were detected ARGs in soil samples by PCR. The results of this study can explain antibiotics contamination status that help we understand occurrence, fate, and their resistance gene from the two swine farming system (Figure 17).

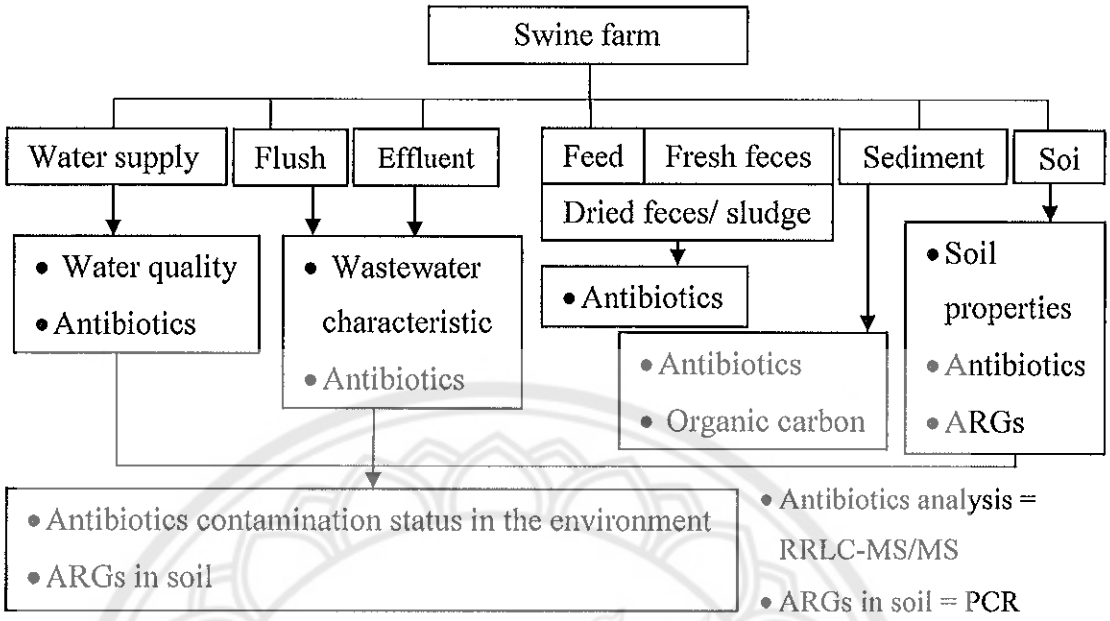


Figure 17 Overall process of the study

Materials and Tools

1. Oasis HLB cartridge 6cc, 500 mg
2. SAX cartridges 6cc, 500 mg
3. SEP-PAK reservoir adaptor
4. Male/Male luer fitting 100/BX
5. SPE tube adaptor
6. Vacuum pump
7. Erlenmeyer flask (1000 mL)
8. Centrifuge and rotors
9. Freeze-dryer
10. Nitrogen gas
11. Ultrasonicator
12. Evaporator
13. Refrigerator and freezer (-20 ^{o}C)
14. GF/F paper (0.22 m)
15. Vortex
16. Amber vial (2 mL)
17. Vial rack for 2 mL

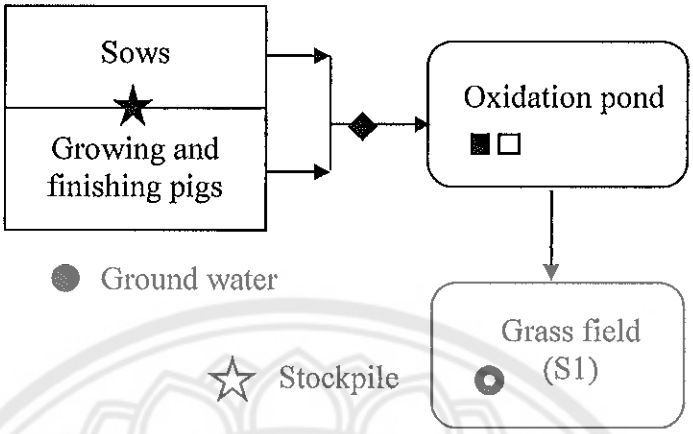
18. Syringe filler (0.22 m)
19. Parafilm
20. Foil paper
21. Centrifuge tube (30 mL)
22. Glass tube (30 mL)

Samples and sampling methods

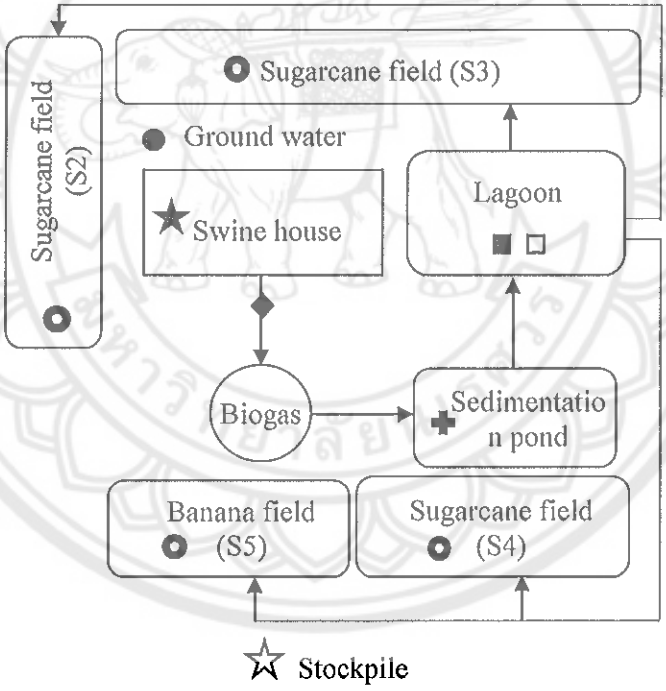
Various samples were collected in July, 2016 from the two swine farms. On the two farms, the collected samples included water supply from storage tank, fresh feces and flush water from swine houses, effluent and sediment from the oxidation pond and lagoon, dried feces or dried sludge from stockpiles, and soil from agricultural fields. Fresh feces samples from typical farm were taken by randomly collecting from different swine houses and then combining into one composite sample. For commercial farm, fresh feces were composited from 5 to 6 grab samples and then combining into one composite sample. The flush waters were sampled at washing time, composited from 5 to 6 grab samples and then combining into one composite sample. The effluent samples were composited from 5 to 6 grab samples. Dried feces and dried sludge were collected from stockpile and soil samples were collected at a depth of 20 cm below the surface soil. Ten discrete subsamples were collected, and composite samples were prepared by mixing equal quantities of subsamples and selected by the quadripartite method. The swine layout of the two swine farms and the sampling site were shown in Figure 18.

1,000 mL of water supply, 200 mL of flush water, and 500 mL of effluent were collected using the brown amber bottles which were rinsed with sample water before collection. All the water samples collected were adjusted to pH 3 using 4 M H_2SO_4, added with methanol (5% v/v) to inhibit microbial activity and then transported to the laboratory in a cooler. 500 g of feed, feces, sludge, sediment, and soil samples were collected and stored in 1 L brown glass bottles and preserved by adding with 2 g of sodium azide. Upon arrival at the laboratory, the samples were immediately stored at 4 °C. Before being analyzed, the solid samples were freeze-dried, sieved through a 0.5 mm pore size and then kept at -18 °C in the dark until extraction (Zhou et al., 2012).

Typical swine farm



Commercial swine farm



Wastewater flow
Groundwater sample
Flush water sample
Wastewater sample
Sediment sample
Fresh feces sample
Dried feces sample
Sludge sample
Soil sample

Figure 18 Layout and sampling site of the two swine farms

Analytical methods

Analytical methods of the samples from the two swine farms were analyzed using chemical methods for wastewater characteristics, soil properties, heavy metal in soil, and antibiotic concentrations. The details as below.

1. Analysis of wastewater characteristics

The parameters of wastewater analysis were temperature, pH, total suspended solid (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total kjeldahl nitrogen (TKN) that followed *Thailand's swine wastewater* parameters. These parameters were detected using equipment or method as showed in Table 9.

Table 9 Wastewater characteristics parameters for swine farm in Thailand

Parameters	Equipment/method
pH	pH meter
Temperature	Thermometer
TSS	Glass Fiber Filter Disc at 103°C - 105°C
COD	Potassium Dichromate Digestion
BOD	Azide Modification
TKN	Kjeldahl

2. Soil property analysis

The parameters of soil property analysis were soil texture, pH value, organic matter (OM) value, total nitrogen (N) content, available phosphorus (P) content, and available potassium (K) content. These parameters were detected using equipment or method as showed in Table 10.

Table 10 Soil properties parameters and method

Parameters	Equipment/method
Soil texture	Hydrometer method
pH	pH meter
OM	Walkley-Black
Total N	Kjeldahl method
Available P	Bray-II
Available K	Extracted by ammonium acetate

3. Antibiotics analysis

In this study, the method was used following the laboratory direction of State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry (GIG), Chinese Academy of Sciences, Guangzhou, China. The details as below.

3.1 Sample extraction

3.1.1 Liquid samples

The collected liquid samples (1,000 mL of water supply, 200 mL of flush water, and 500 mL of effluent) were extracted by solid phase extraction (SPE), show in Figure 20. The liquid samples were filtered through glass fiber filters to remove suspended solids (SS) and then filtered liquid samples were spiked with 100 μL of the internal standards (IS) for chemical analysis. The IS were showed in table 11.

The liquid samples were passed through Oasis HLB cartridges (6 mL, 500 mg) under vacuum at a flow rate of 5-10 mL/min. The target compounds were eluted with 12 mL methanol and then the eluates were evaporated to near dryness under a gentle stream of nitrogen and redissolved in 1 mL of methanol. After filtration through a 0.22 m membrane to remove particles, the final extract was transferred to a 2 mL amber vial and stored at -18 °C until RRLC-MS/MS analysis. Just prior to the RRLC-MS/MS analysis, 100 μL aliquot of each sample extract was evaporated and reconstituted in a mixed solvent (methanol: 0.2% formic acid and 2 mM ammonium acetate, 30:70, v/v) (Zhou et al., 2012).

3.1.2 Solid samples

The solid samples (0.5 g of freeze-dried feces, sludge, 2 g of freeze-dried sediment, soil, feed, and all of each SS) were extracted by ultrasonication, show in Figure 19. The solid samples were weighted into a 30 mL glass tube, followed by addition of 100 μL of the IS for chemical analysis. Then the samples were mixed and placed in a refrigerator at 4 ◦C overnight. The samples were extracted with 10 mL acetonitrile and 10 mL citric acid was added into glass tube followed by mixing on a vortex mixer for 1 min, ultrasonicated for 15 min and centrifuged at 3,500 rpm for 10 min. The supernatant was piped into a 200 mL round-bottom flask. The extraction process was repeated twice and the supernatants from the three extractions were combined. The extract in the round-bottom flask was evaporated at 50 °C, and diluted to 200 mL with MilliQ water. The extracts were purified by passing through tandem SAX cartridges (6 mL, 500 mg) and HLB cartridges (6 mL, 200 mg) under vacuum at a flow rate of 5-10 mL/min. The elution and reconstitution conditions were the same as those described in Section 3.1.1.

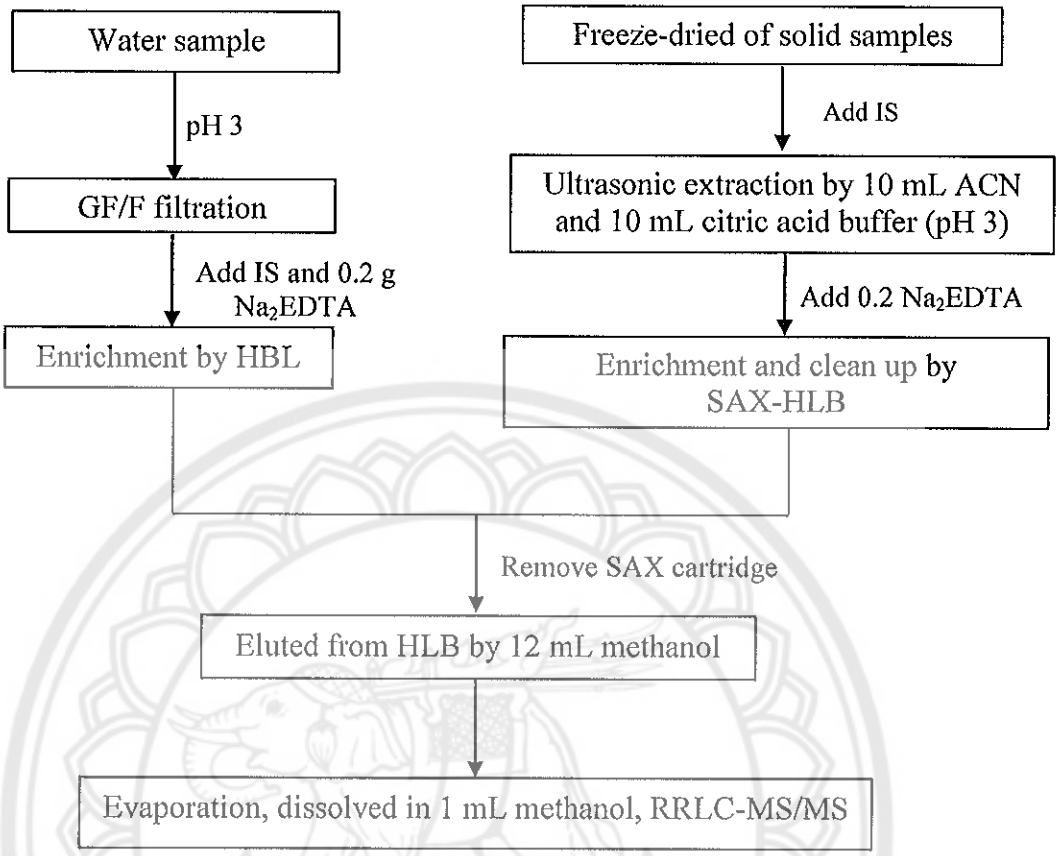


Figure 19 Sample preparation procedure diagram

Source: Zhou et al., 2012

3.1.3 Chemical

The chemicals in this study included 41 antibiotics belonging to eight groups of widely used in swine production of Thailand, comprising: lincomycin (LIN), trimethoprim (TMP), sulfamerazine (SMR), sulfameter (SM), sulfamethazine (SMZ), sulfacetamide (SCM), sulfaguanidine (SG), sulfanilamide (SA), sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SPD), sulfamonomethoxine (SMM), sulfachloropyridazine (SCP), sulfamethoxazole (SMX), sulfadimethoxine (SDM), sulfadoxine (SDO), sulfisoxazole (SX), sulfaquinoxaline (SQX), ciprofloxacin (CFX), marbofloxacin (MAR), fleroxacin (FL), norfloxacin (NFX), carbadox (CAR), ofloxacin (OFX), ormetoprim (OMP), pefloxacin (PEF), lomefloxacin (LFX), danofloxacin (DAN), enrofloxacin (EFX), sarafloxacin (SAR), difloxacin (DIF),

erythomycin (ETM), clarithromycin (CRM), leucomycin (LCM), roxithromycin (RTM), Oleandomycin (ODM), tylosin (TYL), tetracycline (TC), methacycline (MC), narasin (NRS) and monensin (MNS) being selected as the target analytes.

The target antibiotics were analyzed using RRLC-MS/MS, Agilent Liquid Chromatography 1200 series RRLC system coupled to an Agilent 6460 triple quadrupole MS equipped with an electrospray ionization (ESI) source (Agilent, Palo Alto, CA, USA) in multiple-reaction monitoring (MRM) mode. Nitrogen gas was used as the drying and collision gas. LC and MS parameters were measured using an Agilent Eclipse Plus-C18 (100 mm×2.1 mm, 1.8 m) column with its corresponding pre-column filter (2.1 mm, 0.2 m). The column temperature was set at 40 °C. Gas temperature and gas flow were set at 325 °C and 6 L/min, respectively. Sheath gas flow and sheath gas temperature were set at 11 L/min and 350 °C. The injection volume for each sample was 5 μL.

Table 11 Detail of antibiotics and Internal Standard (IS) chemicals

No.	Antibiotics	IS	M.W.	R.T.
Sulfonamides				
1	Sulfamerazine	Sulfamerazine-D4	264.31	3.860
2	Sulfachlorpyridazine	Sulfamerazine-D4	284.72	7.826
3	Sulfameter	Sulfamethazine-13C6	280.30	5.618
4	Sulfamethazine	Sulfamethazine-13C6	278.33	5.272
5	Sulfacetamide	Sulfamethazine-13C6	214.24	2.203
6	Sulfaguanidine	Sulfamethazine-13C6	214.24	1.098
7	Sulfanilamide	Sulfamethazine-13C6	172.20	1.283
8	Sulfadiazine	Sulfamethazine-13C6	250.28	2.582
9	Sulfathiazole	Sulfamethazine-13C6	255.32	3.093
10	Sulfapyridine	Sulfamethazine-13C6	249.29	3.361
11	Sulfamonomethoxine	Sulfamethoxazole-d4	280.30	7.457
12	Sulfamethoxazole	Sulfamethoxazole-d4	253.28	8.903
13	Sulfadimethoxine	Sulfamethoxazole-d4	310.33	11.33

Table 11 (cont.)

No.	Antibiotics	IS	M.W.	R.T.
14	Sulfadoxine	Sulfamethoxazole-d4	310.33	9.006
15	Sulfisoxazole	Sulfamethoxazole-d4	267.30	9.903
16	Sulfaquinoxaline	Sulfamethoxazole-d4	300.37	11.420
Tetracyclines				
17	Tetracycline	Thiabendazole-d4	444.44	6.054
18	Methacycline	Meclocycline	442.42	10.017
Lincosamides				
19	Lincomycin	Lincomycin-3D	406.54	2.964
Macrolides				
20	Erythomycin-H_2O	Erythromycin-13C-d3	733.94	12.976
21	Leucomycin	Erythromycin-13C-d3	686.81	13.147
22	Roxithromycin	Erythromycin-13C-d3	837.05	13.577
23	Oleandomycin	Erythromycin-13C-d3	687.83	13.662
24	Clarithromycin	Erythromycin-13C-d3	747.95	15.333
25	Tylosin	Sulfamethazine-13C6	916.10	12.609
Fluoroquinolones				
26	Ciprofloxacin	Ciprofloxacin-d8	331.35	5.738
27	Marbofloxacin	Ciprofloxacin-d8	370.41	4.510
28	Norfloxacin	Ciprofloxacin-d8	319.34	5.252
29	Danofloxacin	Ciprofloxacin-d8	357.37	10.360
30	Difloxacin	Ciprofloxacin-d8	399.39	8.718
31	Enrofloxacin	Ciprofloxacin-d8	359.40	7.237
32	Fleroxacin	Ciprofloxacin-d8	369.34	5.103
33	Ofloxacin	Ciprofloxacin-d8	361.37	5.334
34	Pefloxacin	Ciprofloxacin-d8	333.36	5.573
35	Sarafloxacin	Ciprofloxacin-d8	385.40	8.514
36	Lomefloxacin	Ciprofloxacin-d8	351.35	6.426
37	Carbadox	Thiabendazole-d4	262.22	5.046

Table 11 (cont.)

No.	Antibiotics	IS	M.W.	R.T.
Ionophores				
38	Narasin	Thiabendazole-d4	765.04	19.130
Diaminopyrimidines				
39	Trimethoprim	Trimethoprim-3d	290.32	4.143
40	Ormetoprim	Thiabendazole-d4	274.32	5.251
Other				
41	Monensin	Thiabendazole-d4	670.88	21.879

Note: IS = Internal standard; M.W. = Molecular weight; R.T. = Retention time;
Source: U.S. National library of medicine chemidplus advanced.
(http://chem.sis.nlm.nih.gov/chemidplus/), 28 September, 2017

3.1.4 Quantification and validation

The internal standard method were used to determine antibiotic concentrations in the samples for this study. The strict quality control procedures were important and necessary. For each a set of samples to be analyzed, a solvent blank, a procedure blank and an independent check standard (100 µg/L standard solution) were run in sequence to check for carry-over, background contamination, and system performance (Zhou et al., 2012). The quantitative values of each target compound were reported with the same retention time as its calibration standard (within ±5%) and the same ion ratios (within ±20%). Approximately every twenty injections have must to check for independent standard. The measurement of antibiotic concentrations was required to be within 20% of the expected value (Monteiro et al. 2015). The minimum detectable amount of an analytes from the environmental matrix were determined with limits of detection (LOD) and of quantification (LOQ) in MRM mode with a signal-to-noise (S/N ratios) of 3 and 10, respectively. (Zhou et al., 2012).

3.1.5 Calibration

Calibration curves were constructed with standard concentration levels at 1.0, 5.0, 10, 50, 100, 200 µg/l and excellent linearity was achieved in the

concentration ranges with correlation coefficients higher than 0.99 ($R^2 > 0.99$) for all validation batches (Liu et al., 2011). The recovery (%), limit of detection (LOD), and limit of quantitation (LOQ) were shown in Table 12.

Table 12 % Recovery, LOD, and LOQ

Antibiotics	Spiked concentrations (µg/L)				LOD	LOQ
	10	50	100	200	ng/L	ng/L
Lincomycin	82.5	112.1	103	98.6	0.14	0.41
Trimethoprim	102.4	102	96.8	100.7	0.06	0.18
Sulfamerazine	102.1	101.8	96.7	100.7	0.06	0.18
Sulfameter	114.2	94.3	91.2	102.5	0.08	0.25
Sulfamethazine	99.9	102.9	96.8	100.6	0.06	0.002
Ciprofloxacin	112.5	93.7	97.5	101	0.18	0.56
Erythromycin-H_2O	91.7	104.6	95.9	100.8	0.08	0.25

Note: LOD = Limit of detection, and LOQ = Limit of quantitation

Fate of antibiotics from traditional and commercial swine farms

Partitioning coefficient (K_d) is the sorptive exchange of chemicals between two phase such as a water phase and a solid phase (sediments or suspended solid)

Sediment/aqueous partition coefficient (K_d) for each chemical were calculated using the relationship:

$$K_d = C_s/C_{aq} \quad (1)$$

Where C_s is the concentration of veterinary antibiotics adsorbed by sediments or suspended solid in ng/g,

C_{aq} is the concentration of veterinary antibiotics in aqueous phase in ng/L

In addition, Kd depends on fraction of organic carbon ($f$oc) then Kd related to normalized organic carbon content (Koc) with relationship according to equation 2: The Koc values were calculated by using the expression in equation

$$K_{oc} = K_d \times 100/\%TOC \quad (2)$$

Octanol-water partition coefficient (K_{ow}), and distribution ratio (Dow)

The mobility of antibiotics in soil was determined with octanol-water partition coefficient (K_{ow}), as show in equation

$$K_{ow} = \frac{\text{(Solute) octanol}}{\text{(Solute) water}}$$

Antibiotic Resistance Genes (ARGs)

1. Samples collection

The collected samples including, surface soil samples (0-20 cm) of agricultural fields from typical farm and commercial farm were taken by randomly collecting soil sample. After that combining into one composite sample. Five composite samples were collected in each separate area from commercial farm. In addition, the control samples from each farm were collected from agricultural soil without manure application nearby. Each composite sample was placed in a one plastic bag and transported back to Naresuan University Phitsanulok, Thailand in coolers containing ice. The final samples about 1 kg in each composite sample were used for antibiotic resistant genes analysis. Prior to analysis, all samples were air dried at ambient temperature in the dark, ground and homogenized by sieving through a 2 mm of stainless steel sieve after removing stones and residual roots for DNA extraction, the remaining soils were frozen at -20 °C within three months.

2. DNA extraction

DNA samples were extracted from 250 mg of soil with a commercial kit (GenElute™ Soil DNA Isolation Kit product from Sigma-Aldrich, Thailand). The extraction method was conducted following the manufacturer's protocol. Finally, DNA bands were checked on agarose gel electrophoresis.

2.1 Materials, Chemicals and Tools

2.1.1 GenElute™ Soil DNA Isolation Kit

2.1.2 Soil samples

2.1.3 Microcenrifuge

2.1.4 Flat-bed vortexer

2.1.5 Parafilm

2.1.6 Erlenmeyer flask (500 mL)

2.1.7 Micro centrifuge tubes (1.7 ml)

2.1.8 DNase-free water

2.1.9 Flatbed vortex

2.1.10 96-100% ethanol

2.1.11 Ice

2.1.12 Ice box

2.1.13 Agarose

2.1.14 0.5 M EDTA

2.1.15 TAE (1X)

2.1.16 Orange loading dye

2.1.17 Pipettes (1000, 20-200 and 1-10 μL)

2.1.18 Pipette tips

2.1.19 Gel electrophoresis

2.1.20 Gel tray and comb

2.1.21 Microwave

2.1.22 Plastic containers with lids

2.1.23 Refrigerator and freezer (-20°C)

2.1.24 Latex or nitrile gloves

2.1.25 Microcentrifuge tube rack

3. Polymerase chain reaction (PCR)

3.1 Materials, Chemicals and Tools

3.1.1 Primers (*tet*M, *tet*O, *erm*A, *erm*B, *qnr*A and, *qnr*B)

3.1.2 Template (extracted DNA from sample)

3.1.3 PCR master mix (GeneDireX)

3.1.4 DNase RNase free water

3.1.5 DNA template (The extracted DNA from 2.)

3.1.6 Thermo Cycler

3.1.7 PCR microcenrifuge

3.1.8 Flat-bed vortexer

3.1.9 Parafilm

3.1.10 Centrifuge

3.1.11 Ice and ice box

3.1.12 Erlenmeyer flask (500 mL)

3.1.13 1.7 mL DNase free microcentrifuge tube

3.1.14 DNAse-free microcentrifuge tube

3.1.15 96-100% ethanol

3.1.16 Agarose

3.1.17 0.5 M EDTA

3.1.18 TAE (1X)

3.1.19 Orange loading dye

3.1.20 Pipette

3.1.21 Pipette tip

3.1.22 Gel electrophoresis units

3.1.23 Gel tray and comb

3.1.24 Microwave

3.1.25 Refrigerator and freezer (-20°C)

3.1.26 PCR microcentrifuge tube rack

3.2 Primes

Primers for PCR amplification of six different genes were either selected based on the published sequences available in Genbank. The target genes included tetracycline resistance genes (*tet*M and *tet*O), erythromycin resistance genes conferring resistances to macrolide-lincosamides-streptogramin (MLS genes: *erm*A and *erm*B) and quinolone resistance genes (*qnr*A and *qnr*B). The specific primer pair and sequences were listed in Table 14. Working solution stocks of primer were prepared by combining 198 µL of molecular grade water with 2 µL of the designated primer in a sterile 0.5 mL microcentrifuge tube. They can be made ahead and stored in the freezer.

3.3 PCR reactions

DNA of soil samples was amplified using thermo cycler PCR machine in a 25 μL reaction volume. Quantities given were for one reaction tube, 12.5 μL of PCR master mix, 1 μL of each primer, 1 μL of DNA template and 9.5 μL DNase RNase free water, with the following PCR cycling conditions. Multiply amount needed for one reaction tube by the number of samples to be run. Add one negative control for each gel.

3.4 PCR cycling conditions

Before setting up the PCR reaction must turn on the thermal cycler. Then put the PCR reaction tubes in the wells of thermal cycler and close the lid. Start program, with the following PCR conditions as show in Table 13.

Table 13 PCR cycling conditions

Step/Target genes	***tet*O, M, *erm*A, B**	***Qrn*A, B**
Pre-denaturing	94°C/5 min	94°C for 2 min
Step 1	94°C/1.5 min	94°C/45 sec
Step 2	55°C/1 min,	53°C/45 sec
Step 3	72 °C/1 min	72°C/1 min
Final extension	72°C/5 min	72°C/5 min
Cycles	35	30
Hold	4°C/infinity	4°C/infinity

Table 14 Primers employed in the present study for PCR

Primer	Sequences (5'→3')	Annealing Temp (°C)	Size (bp)	References
*tet*M-FW	GTG GAC AAA GGT ACA ACG AG	55	406	Ng et al., 2001
*tet*M-RW	CGG TAA AGT TCG TCA CAC AC			
*tet*O-FW	AAC TTA GGC ATT CTG GCT CAC	55	515	Ng et al., 2001
*tet*O-RW	TCC CAC TGT TCC ATA TCG TCA			
*erm*A-FW	CCC GAA AAA TAC GCA AAA TTT CAT	55	590	Malhotra-Kumar, S. et al., 2005
ermA-RW	CCC TGT TTA CCC ATT TAT AAA CG			
*erm*B-FW	TGG TAT TCC AAA TGC GTA ATG	55	745	Malhotra-Kumar, S. et al., 2005
ermB-RW	CTG TGG TAT GGC GGG TAA GT			
*qnr*A-FW	CAG CAA GAG GAT TTC TCA CG	53	630	Ciesielczuk H. et al., 2013
qnrA-RW	AAT CCG GCA GCA CTA TTA CTC			
*qnr*B-FW	GGC TGT CAG TTC TAT GAT CG	53	488	Ciesielczuk H. et al., 2013
*qnr*B-RW	GAG CAA CGA TGC CTG GTA G			

3.5 Agarose gel electrophoresis

The PCR products were electrophoresed on TAE agarose gel buffer. Agarose gels were prepared by adding 0.4 g agarose powder to 40 ml of 1x TBE (Tris-Borate-EDTA) buffer (Lee et al., 2012). The powder solution was boil in a microwave oven until the agar solution was completely dissolved, and then carefully remove it from microwave oven. The solution was cooled down to 55°C, add 0.5 µl of ethidium bromides and mixed gently. The solution was poured into a casting tray and comb. The gel solution was set, carefully pull out the combs and remove the tape and then were moved in the electrophoresis chamber. 10 µl volume of each sample were loaded onto a gel. Orange DNA loading dye was loaded in the first well of the gel as marker. However, the distil water was loaded into the gel as negative control, then the gel was run at 100 V for 35 minute. The DNA bands were checked under UV light. The photos were taken from the gels in a dark room by using digital camera (Wang, & Wen, 2010).

3.6 Gel purification and sequence analysis

Gel purification, the target DNA bands were cut out from the TAE agarose gel and purified them using a HiYield™ Gel/PCR fragments extraction kit (RBCBioscience) following manufacturer's instructions (Jantafong et al., 2015). All purify DNA bands of melting gel were directly sequenced and measured of DNA concentration using a NanoDrop™ spectrophotometer prior to sequence analysis. The results were compared sequence analysis in the BLAST databases available from NCBI.

CHAPTER IV

RESULTS

The study of occurrence and fate of antibiotics and antibiotic resistance genes (ARGs) from typical and commercial swine farm was determined wastewater characteristics, soil properties, heavy metal in soils, antibiotic concentrations in various samples and ARGs in agricultural soils. Water supply in swine farm, flush water, effluent, (aqueous, suspended solids phase), sediment from an oxidation pond and lagoon, sludge from a biogas system, swine feeds, fresh feces, dried feces or dried sludge and agricultural soil samples were collected from one typical swine farm and one commercial swine farm in Phitsanulok province, Thailand. The chemical analysis was used to determine effluent characteristics, soil properties, heavy metal in soils and antibiotic concentrations. Molecular analysis was used to determine ARGs in agricultural soils. The results of this study were shown as below.

Site and system description

One typical and one commercial farms with different wastewater management systems were selected for this study. The two swine farms, representing typical swine feeding operations in Phitsanulok province, are located in Mueang and Bang Rakam district. The typical farm consists of several buildings for piglets, growing and finishing pigs and sows. This farm accommodated 150-pigs small scale, including 40 piglets, 100 growing and finishing and 10 sows. The swine houses were flushed daily with water supply and the mixed flush water was directly discharged into an oxidation pond. Wastewater in the pond was partially applied onto grass field nearby the farm. For commercial farm, it was designed for 750-pigs medium scale with evaporative cooling system. The swine houses were flushed daily with water supply and the flush water was treated in a biogas system followed by a lagoon. The lagoon wastewater was partially applied onto the sugarcane and banana fields nearby the farm.

Wastewater characteristic

Wastewater characteristic of two swine farms were measured the parameter, namely pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solid (TSS), Total Kjeldahl Nitrogen (TKN) and Total Organic Carbon in sediment (TOC). The results were shown as below.

1. pH

pH values of water supply, flush water and effluent from typical swine farm and commercial swine farm are shown in Table 15. Water supply, pH values were found of 7.95±0.01 from typical farm and 7.95±0.03 from commercial farm. Flush water, pH values were found of 6.73±0.17 from typical farm and 8.68±0.03 from commercial farm. For the effluent, pH values were found of 7.53±0.05 from typical farm and 8.08±0.03 from commercial farm. In addition, the pH values in both typical farm and commercial farm were within the criteria of Thailand's standard.

2. Temperature

Temperature of effluent from typical and commercial swine farm are shown in Table 15. Water supply, temperatures were found of 29.5±0.50 °C from typical farm and 30.0±1.2^{0} °C from commercial farm. For flush water, temperatures were found of 31.0±0.80 °C from typical farm and 32.5±1.40 °C from commercial farm. Effluent, the temperatures were found of 32.0±1.50 °C from typical farm and 32.5±0.02 °C from commercial farm.

3. Chemical Oxygen Demand (COD)

COD of flush water and effluent from typical swine farm and commercial swine farm are shown in Table 15. Flush water, the average COD were found of 1,038.33±40.72 mg L^{-1} from typical farm and 1,576.00±5.29 mg L^{-1} from commercial which were over the criteria of Thailand's standard. For effluent, they were found of 237.67±2.52 mg L^{-1} from typical farm and 386.67±4.16 mg L^{-1} from commercial farm which were within the criteria of Thailand's standard. In addition, the average COD from commercial farm were higher than those from typical farm.

4. Biological Oxygen Demand (BOD)

BOD of flush water and effluent from typical swine farm and commercial swine farm are shown in Table 15. Flush water, the average BOD were found of 597.00±1.73 mg L^{-1} from typical farm and 1,035±21.79 mg L^{-1} from commercial farm

which were over the criteria of Thailand's standard. For effluent, the average BOD were found of 122.83±2.57 mg L^{-1} from typical farm which were over the criteria of Thailand's standard. Moreover, the average BOD in the lagoon effluent from commercial farm were found of 33.83±1.61 mg L^{-1} which were within the criteria of Thailand's standard.

5. Total Suspended Solid (TSS)

TSS of water supply, flush water and effluent from typical swine farm and commercial swine farm are shown in Table 15. water supply, the average TSS were found of 38.17±0.29 g L^{-1} from typical farm and 27.50±0.50 g L^{-1} from commercial farm which were within the criteria of Thailand's standard. Flush water, the average TSS were found of 364.33±21.57 g L^{-1} from typical farm and 542.33±5.86 g L^{-1} from commercial farm which were over the criteria of Thailand's standard. For effluent, the average TSS were found 46.67±4.16 g L^{-1} from typical farm and 36.23±1.74 g L^{-1} from commercial farm which were within the criteria of Thailand's standard.

6. TDS (Total Dissolve Solid)

TDS of water supply, flush water and effluent from typical swine farm and commercial swine farm are shown in Table 15. Water supply, the average TDS were found of 0.28±0.01 g L^{-1} from typical farm and 2.52±0.02 g L^{-1} from commercial farm. Flush water, the average TDS were found of 8.93±0.02 g L^{-1} from typical farm and 11.14±0.14 g L^{-1} from commercial farm. For effluent, the average TDS were found 4.52±0.03 g/L from typical farm and 3.89±0.02 g L^{-1} from commercial farm.

7. Total Kjeldahl Nitrogen (TKN)

TKN of water supply, flush water and effluent from typical swine farm and commercial swine farm are shown in Table 15. Water supply, the average TKN were found of 0.57±0.04 mg L^{-1} from typical farm and 0.53±0.10 mg L^{-1} from commercial farm which were within the criteria of Thailand's standard. Flush water, the average TKN were found of 180.38±1.13 mg L^{-1} from typical farm which were within the criteria of Thailand's standard. For commercial farm, the average TKN in flush water were found of 752.00±1.73 mg L^{-1} from which were over the criteria of Thailand's standard. For effluent, the average TKN were found of 56.66±1.26 mg L^{-1} from typical farm, which were within the criteria of Thailand's standard. Furthermore, the average

TKN in the effluent from commercial farm were found of 204.83±0.76 mg L^{-1} which were over the criteria of Thailand's standard.

8. Total Organic Carbon (TOC) in sediment

TOC of sediment in an oxidation pond from typical swine farm and TOC of lagoon sediment from commercial farm are shown in Table 16. The average TOC were found of 24.67±0.47 % from typical farm and 13.35±4.64 % from commercial farm. In addition, the average TOC from typical farm were higher than those from commercial farm.

Table 15 Characteristics of swine wastewater quality parameters of aqueous samples (mg L^{-1})

Parameter	Groundwater*		Flush water*		Wastewater*		Thailand swine wastewater quality standard	
	Traditional	Commercial	Traditional	Commercial	Traditional	Commercial	Large scale	Small scale
pH	7.95±0.01	7.95±0.03	6.73±0.17	8.68±0.03	7.53±0.05	8.08±0.03	5.5-9.0	5.5-9.0
Temp (°C)	29.50 ± 0.50	30.00±1.20	31.00±0.80	32.50±1.40	32.00±1.50	32.50±±0.02	-	-
COD (mg/L)	4.56±1.52	7.89±0.43	1,038.33±40.72	1,576±5.29	237.67±2.52	386.67±4.16	300	400
BOD (mg/L)	0.35±0.04	0.29±0.02	597.00±1.73	1,035±21.79	122.83±2.57	33.83±1.61	60	100
TSS (g/L)	38.17±0.29	27.50±0.50	364.33±21.57	542.33±5.86	46.67±4.16	36.23±1.74	150	200
TDS (g/L)	0.28±0.01	2.25±0.02	8.93±0.02	11.14±0.14	4.52±0.03	3.89±0.02	-	-
TKN (mg/L)	0.57±0.04	0.53±0.10	180.38±1.13	752±1.73	56.66±1.26	204.83±0.76	120	200

Note: * Mean ± standard deviation (n=3). Temp, temperature; COD, chemical oxygen demand; BOD5, biochemical oxygen demand; TSS, Total Suspended Solid; TDS, Total Dissolve Solid; TKN, Total Kjeldahl Nitrogen; TOC, Total Organic Carbon.

Table 16 Characteristics of swine wastewater sediments (g/kg dry weight)

Sample/Parameter	Farm type	pH*	TOC*	TN*	TP*	TK*
Oxidation pond sediment	Typical	7.53±0.23	24.67±0.47	1.84±0.05	4.47±0.01	1.21±0.16
Lagoon sediment	Commercial	7.50±0.05	13.35±4.64	0.95±0.31	2.19±0.25	0.65±0.05

Note: * Mean ± standard deviation (n=3). TOC, Total Organic Carbon; TN, Total Nitrogen; TP, Total Phosphorus; TK, Total Potassium

Soil properties

Soil properties of agricultural soil samples from the two swine farms were measured the parameter, namely soil texture, soil pH, Organic Matter (OM), Nitrogen (N), Phosphorus (P) and Potassium (K). The results were shown as below and Table 17.

1. Soil texture

Soil textures were sandy clay loam from typical swine farm and silty clay from commercial swine farm. The particle size distributions were found 63% sand, 70% silt and 30% clay from typical swine farm and 50% sand, 50% silt and 55% clay from commercial swine farm.

2. Soil pH

Soil pH values of agricultural soil from typical and commercial swine farm are shown in Table 17. The pH value from S1, S2, S3, S4 and S5 samples were found of 6.84±0.01, 8.01±0.01, 8.05±0.01, 7.98±0.04 and 8.01±0.01, respectively.

3. Organic Matter (OM)

OM levels of agricultural soil from typical and commercial swine farm are shown in Table 17. The average OM from S1, S2, S3, S4 and S5 samples were found of 42.53±0.82, 40.80±0.44, 51.85±0.59, 41.79±0.28 and 42.32±0.45%, respectively.

4. Nitrogen (N)

N levels of agricultural soil from typical and commercial swine farm are shown in Table 17. The average N from S1, S2, S3, S4 and S5 samples were found of, 2.13±0.04, 2.31± 0.02, 2.33±0.06, 2.28±0.03 and 2.26±0.13 mg kg^{-1}, respectively.

5. Phosphorus (P)

P levels of agricultural soil from typical and commercial swine farm are shown in Table 17. The average P from S1, S2, S3, S4 and S5 samples were found of 4.47±0.58, 5.82± 0.02, 5.51±0.05, 5.50±0.02 and 5.29±0.08 mg kg^{-1}, respectively.

6. Potassium (K)

K levels of agricultural soil from typical and commercial swine farm are shown in Table 17. The average K from S1, S2, S3, S4 and S5 samples were found of, 17.07±1.10, 28.42±1.24, 53.88±2.62, 40.93±23.62 and 40.67±2.49 mg kg^{-1}, respectively.

Table 17 Soil properties

Sample	Soil sample	Soil type	pH*	OM* (%)	N* (mg kg^{-1})	P* (mg kg^{-1})	K* (mg kg^{-1})
Control 1	Grass field	Sandy clay loam	7.05±0.01	4.18±0.51	0.07±0.01	2.50±0.05	0.18±0.03
Control 2	Sugarcane field	Silty clay	7.06±0.02	18.70±0.26	0.87±0.02	2.23±0.06[e]	11.10±0.20
	Grass field	Sandy clay loam	6.84±0.01	42.53±0.82	2.13±0.04	4.47±0.58	17.07±1.10
S2	Sugarcane field	Silty clay	8.01±0.01	40.80±0.44	2.31±0.02	5.82±0.02	28.42±1.24
S3	Sugarcane field	Silty clay	8.05±0.01	51.85±0.59	2.33±0.06	5.51±0.05	53.88±2.62
S4	Sugarcane field	Silty clay	7.98±0.04	41.79±0.28	2.28±0.03	5.50±0.02	40.93±23.62
S5	Banana field	Silty clay	8.01±0.01	42.32±0.45	2.26±0.13	5.29±0.08	40.67±2.49

Note: * Mean ± standard deviation (n=3). S1, grass field soil from typical farm; S2, S3 and S4 sugarcane field soil from commercial farm and S5, banana field soil from commercial farm; OM, Organic Matter; N, Nitrogen; P, Phosphorus and K, Potassium

Heavy metal in soil

Zn, Cu, Pb and Cd levels of agricultural soil from typical and commercial swine farm are shown in Table 18. The average of Zn from control 1, control 2, S1, S2, S3, S4 and S5 samples were found of 0.150±0.02, 0.183±0.03, 0.160±0.04, 0.172±0.07, 0.879±0.50, 0.226±0.01 and 0.253±0.03 mg kg^{-1}, respectively with statistically significant differences ($p \leq 0.05$). The average of Cu from control 1, control 2, S1, S2, S3, S4 and S5 samples were found of 0.133±0.02, 0.483±0.58, 0.138±0.01, 0.138±0.01, 0.153±0.01, 0.153±0.01 and 0.160±0.03 mg kg^{-1}, respectively. The average of Cd from control 1, control 2, S1, S2, S3, S4 and S5 samples were found of 0.052±0.04, 0.067±0.02, 0.012±0.02, 3.184±0.08, 0.608±0.12, 0.453±0.06 and 0.027±0.03 mg kg^{-1}, respectively. For Pb was not found in all the soil samples.

Table 18 Heavy metal in soil samples (ppm)

Sample	Soil sample	Zn	Cu	Cd	Pb
Control 1	Grass field	0.15±0.02	0.13±0.02	0.05±0.04	0.00
Control 2	Sugarcane field	0.18±0.03	0.48±0.58	0.07±0.02	0.00
S1	Grass field	0.16±0.04	0.14±0.01	0.01±0.02	0.00
S2	Sugarcane field	0.17±0.07	0.14±0.01	3.18±0.08	0.00
S3	Sugarcane field	0.88±0.50	0.15±0.01	0.68±0.12	0.00
S4	Sugarcane field	0.23±0.01	0.15±0.01	0.45±0.06	0.00
S5	Banana field	0.25±0.03	0.16±0.03	0.34±0.03	0.00

Note: * Mean ± standard deviation (n=3). S1, grass field soil from typical farm; S2, S3 and S4 sugarcane field soil from commercial farm and S5, banana field soil from commercial farm; Zn, zinc; Cu, copper; Cd, cadmium and Pb, lead; Heavy metal concentration; n = 3

Occurrence of antibiotics

1. Concentrations of antibiotics in swine feed samples

Seven antibiotics including, lincomycin, sulfamerazine, sulfamethazine, sulfameter, ciprofloxacin, erythromycin, and trimethoprim were found in feed from typical swine farm with mean concentrations of 9,191.72±1.15, 1,369.18±1.60, 5,970.40± 2.21, 1,802.84±3.31, 2,782.72±0.01, 825.44±0.05, and 1,712.14 ±1.55 μg kg^{-1}, respectively. Six antibiotics lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in feed from commercial swine farm with mean concentrations of 11,695.81±16.38, 502.73±0.09, 535.64±0.05, 1,102.21±0.07, 1,570.48±0.03, and 474.63±0.91 μg kg^{-1}, respectively (Figure 20).

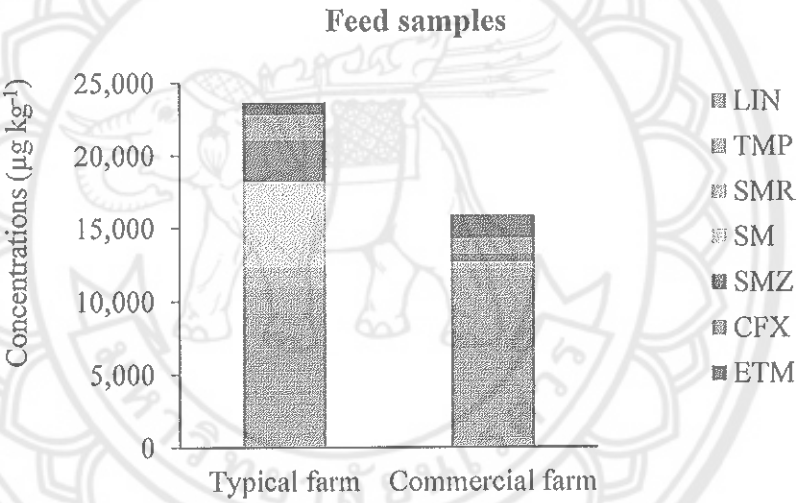


Figure 20 Concentrations of antibiotics in swine feed samples from typical and commercial swine farms. LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CPX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

2. Concentrations of antibiotics in fresh feces samples

Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in fresh feces from typical swine farm with mean concentrations of 40,229.15± 19.71, 3,158.36±0.19, 11,803.98±1.20, 24,594.8±5.65, and 4,833.13±0.87 μg kg^{-1}, respectively. For commercial swine farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in fresh feces with mean concentrations of 22,524.32±1.78, 3,242.96±0.66, 2,349.33±0.44, 11,575.57±0.81, 1,328.08±0.36, and 1,911.87±0.03 μg kg^{-1}, respectively (Figure 21).

3. Concentrations of antibiotics in dried feces and dried sludge samples

Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in dried feces from typical swine farm with mean concentrations of 26,614.38± 21.47, 5,858.58±2.41, 7,658.73±0.61, 21,911.02±4.80, and 6,586.56±2.67 μg kg^{-1}, respectively. For commercial swine farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in dried sludge which was treated by a biogas system with mean concentrations of 4,090.42±1.94, 1,987.7±0.12, 2,292.66±0.31, 14,353.39±1.55, 4,522.49±0.76, and 1,887.45±0.33μgkg^{-1}, respectively (Figure 21).

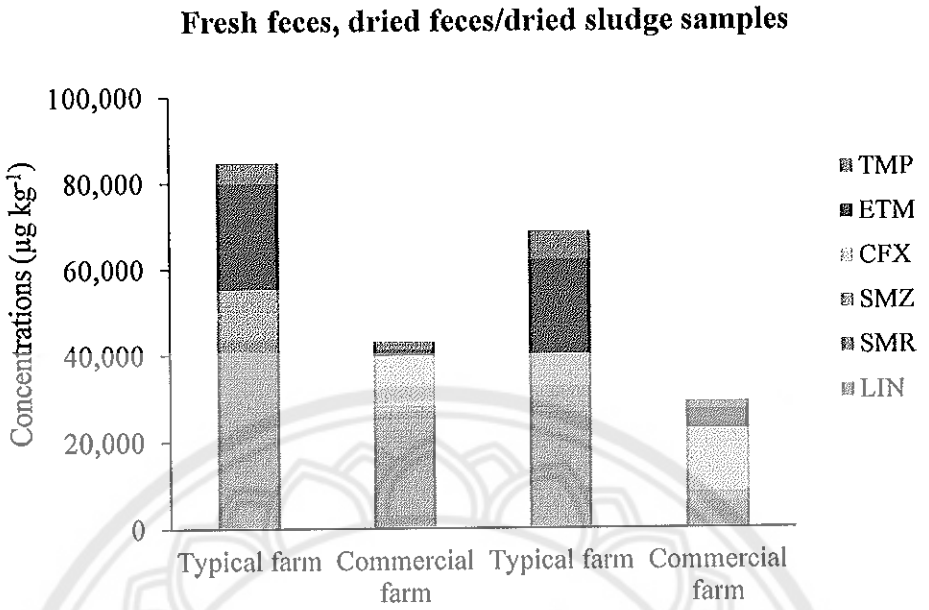


Figure 21 Concentrations of antibiotics in fresh feces, dried feces/dried sludge samples from typical and commercial swine farms. LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CPX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

4. Concentration of antibiotics in flush water samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, and trimethoprim were found in aqueous of the flush water from typical swine farm with mean concentrations of 74.22±11.02, 4.42± 0.01, 51.03±0.60, 21.90±0.23, 54.94±2.72, and 2.44±0.42 ng l^{-1}, respectively. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of the flush water with mean concentrations of 62,918.29±8.96, 5,556.01±0.13, 9,296.18±0.85, 17,472.79±0.69, 3,602.91±0.84, and 4,620.62±0.12 µg kg^{-1}, respectively. For commercial swine farms, lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in aqueous of the flush water with mean concentrations of 351.24±40.56, 0.92±0.04, 598.34±17.27, 64.25±1.04, and 286.34±0.53 ng l^{-1}, respectively. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of the flush water with mean concentrations of 9,395.90±16.67, 788.32±0.05, 865.03±0.73, 3,334.30± 0.95, 5,452.01±1.61, and 1,061.89±0.52 µg kg^{-1}, respectively (Figure 22).

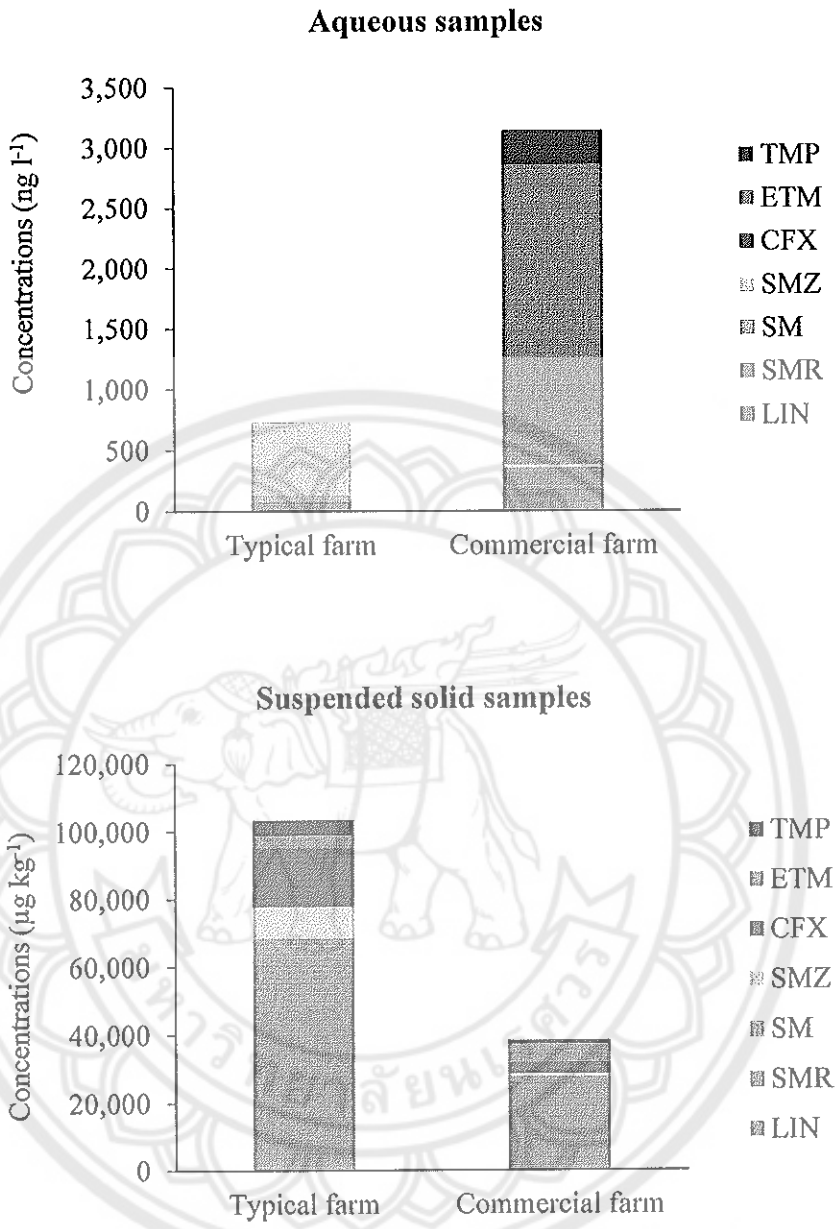


Figure 22 Concentrations of antibiotics in flush water samples from typical and commercial swine farms. LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CPX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

5. Concentrations of antibiotics in effluent samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, and trimethoprim were found in aqueous phase of effluent from typical swine farm with mean concentrations of 120.03±0.05, 1.79±0.25, 51.13±0.03, 773.12±1.82, 9,614.56±1.46, and 1.47±0.05 ng l^{-1}, respectively. Sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS with mean concentrations of 7,594.17±0.06, 31,972.81±0.49, 102,747.26±0.77, 24,553.76±0.56, 154,500.08±12.05, and 8,128.14±0.34 μg kg^{-1}, respectively. Lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in sediment with mean concentrations of 29,624.04±3.12, 518.79±0.12, 3,001.58±0.50, 24,562.79± 1.65, 14,641.29±4.19, 71,123.61±23.28, and 514.69±0.06 μg kg^{-1}, respectively. For commercial swine farm, lincomycin, sulfamerazine, sulfamethazine, and erythromycin were found in aqueous phase of effluent with mean concentrations of 734.46±4.35, 7.26±3.42, 3.72±0.02, and 3.07±0.01 ng l^{-1}, respectively. Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in SS with mean concentrations of 17,275.33±0.20, 1,462.53±0.01, 36,986.96±0.36, 2,997.80±2.53, and 1,540.20±0.36 μg kg^{-1}, respectively. In addition, lincomycin, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in lagoon sediment with mean concentrations of 11,751.66± 0.05, 595.48±1.83, 2,350.70±1.57, 1,677.83±0.13 and 634.66±0.05 μg kg^{-1}, respectively (Figure 23).

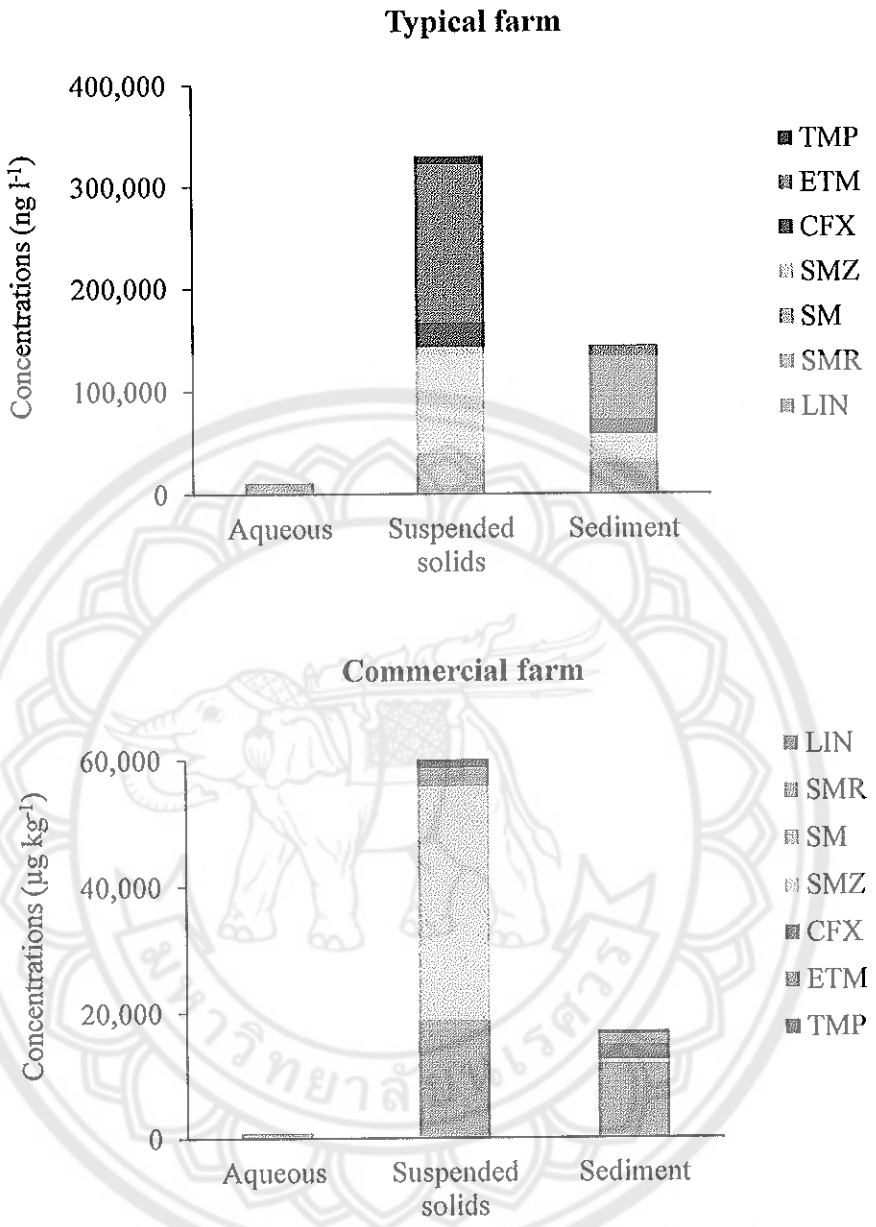


Figure 23 Concentrations of antibiotics in effluent samples from typical and commercial swine farms. LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CPX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

6. Concentrations of antibiotics in the agricultural soil samples

Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in S1 from traditional farm with mean concentration of 4,466.82±11.28, 751.76±0.17, 1,665.75±7.81, 3,593.42±0.05, 5,245.68±1.24 and 1,100.09±0.69 µg/kg, respectively. In commercial farm, lincomycin, sulfamerazine, sulfamethazine, erythromycin and trimethoprim were found in S2 sample with mean concentration of 14,671.65±11.28, 565.39±0.17, 3,903.23±7.81, 137.18±1.24 and 871.17±0.69 µg/kg, respectively. In addition lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found at mean concentration of 18,217.49±15.46, 7,733.69±20.34, 19,372.86±38.75, 1,560.33±0.05, 2,945.15±1.40 and 85,363.39±4.85 µg/kg in S3, 14,134.49±36.97, 626.01±0.38, 3,315.07±6.47, 1,927.07±1.84, 8,066.50±0.27 and 493.3±2.65 µg/kg in S4 and 10,809.47±24.44, 885.26±0.97, 870.4±0.99, 2,961.25±0.45, 405.87±0.48 and 693.86±0.31 µg/kg in S5. (Figure 24).

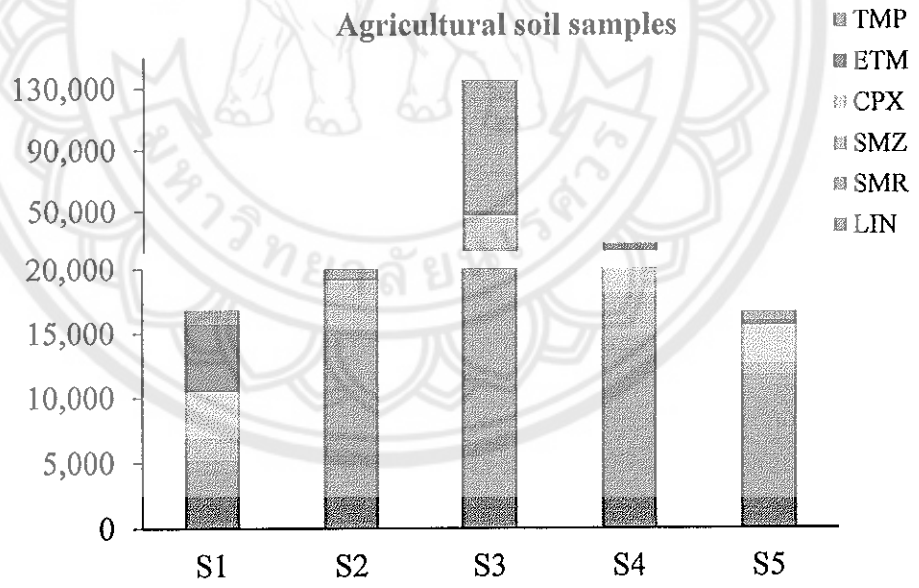


Figure 24 Concentrations of antibiotics in the agricultural soil samples from typical and commercial swine farms. LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CPX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

Correlation between antibiotic concentrations and flush water characteristic parameters

The correlation between antibiotic concentrations and flush water characteristic parameters, including pH, COD, BOD and TSS were observed in aqueous and suspended solids samples of flush water from typical and commercial swine farms and tested with Pearson's correlation.

1. Aqueous samples

The correlation between antibiotics and aqueous phase of flush water characteristic parameters from typical and commercial swine farm (Table 19) showed that the concentrations of lincomycin, sulfamethazine and trimethoprim were high positive correlated to pH with 0.998, 0.994 and 0.995, COD with 0.989, 0.995 and 0.994, BOD with 0.998, 1.000 and 1.000, TSS with 0.979, 0.988 and 0.986 of Pearson's values, respectively. Sulfamerazine and sulfameter were high negative correlated to pH with - 0.989 and -0.995, COD with -0.997 and -0.990, BOD with -1.000 and -0.997, TSS with -0.992 and -0.978 of Pearson's values, respectively. For erythomycin was not correlated to pH, COD, BOD and TSS of Pearson's values.

2. Suspended solids samples

The correlation between antibiotics and suspended solids phase of flush water characteristic parameters from typical and commercial swine farm (Table 20) showed that the concentrations of lincomycin was high positive correlated to pH, COD, BOD and TSS with 0.964, 0.965, 0.968 and 0.957 of Pearson's values, respectively. Sulfamethazine was high negative correlated to TSS with -0.997 of Pearson's values. Erythomycin was high positive correlated to pH with 0.952 of Pearson's values. For sulfamerazine, ciprofloxacin and erythomycin were not correlated to pH, COD, BOD and TSS of Pearson's values.

Table 19 Correlation between antibiotics and aqueous of flush water characteristic parameters from the two swine farms

Water quality parameters	LIN		SMR		SM		SMZ		ETM		TMP	
	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig
pH	0.998**	0.000	-0.989**	0.001	-0.995**	0.000	0.994**	0.001	0.536	0.352	0.995**	0.000
COD	0.989**	0.001	-0.997**	0.000	-0.990**	0.001	0.995**	0.000	0.383	0.524	0.994**	0.001
BOD	0.998**	0.000	-1.000**	0.000	-0.997**	0.000	1.000**	0.000	0.461	0.435	1.000**	0.000
TSS	0.979**	0.004	-0.992**	0.001	-0.978**	0.004	0.988**	0.002	0.357	0.555	0.986**	0.002

Note: ** Correlation is significant at 0.01 level; COD, Chemical Oxygen Demand; BOD, Biological Oxygen Demand and TSS, Total Suspended Solid; LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; ETM, erythromycin and TMP, trimethoprim

Table 20 Correlation between antibiotics and suspended solid of flush water quality parameters from the two swine farms

Water quality parameters	LIN		SMR		SMZ		CFX		ETM		TMP	
	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig
pH	0.964**	0.008	0.972	0.151	0.977	0.137	0.872	0.326	0.952*	0.048	-0.443	0.455
COD	0.965**	0.008	-0.994	0.072	-0.943	0.215	-0.925	0.247	0.841	0.159	-0.599	0.286
BOD	0.968**	0.007	-0.283	0.817	-0.673	0.530	-0.012	0.992	0.900	0.100	-0.526	0.362
TSS	0.957*	0.011	-0.930	0.240	-0.997*	0.048	-0.795	0.415	0.805	0.195	-0.622	0.263

Note: ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; COD, Chemical Oxygen Demand; BOD, Biological Oxygen Demand and TSS, Total Suspended Solid; LIN, lincomycin; SMR, sulfamerazine; SMZ, sulfamethazine; CFX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

Correlation between antibiotic concentrations and effluent characteristic parameters

The correlation between antibiotic concentrations and effluent characteristic parameters such as pH, COD, BOD, TSS and TOC were observed in effluent and sediment samples from typical and commercial swine farms with Pearson's correlation.

1. Aqueous samples

The correlation between antibiotics and aqueous of effluent characteristic parameters from typical and commercial swine farm (Table 21) showed that the concentrations of lincomycin and sulfamerazine were high positive correlated to pH with 0.992 and 0.993, COD with 0.999 and 0.999 of Pearson's values while, they were high negative correlated to BOD and TSS with -0.999 and -0.895. Sulfameter, sulfamethazine, erythomycin and trimethoprim were high negative correlated to pH with -0.985, -0.992, -0.992 and -0.993 while, they were high positive correlated to BOD with 0.999 in these and TSS with 0.920, 0.895, 0.895 and 0.894 of Pearson's values in these, respectively.

2. Suspended solids samples

The correlation between antibiotics and suspended solids of effluent characteristic parameters from typical and commercial swine farm (Table 22) showed that the concentrations of sulfameter was high correlated to pH, COD, BOD and TSS with -0.987, -0.991, 0.993 and 0.926 of Pearson's values. Sulfamethazine and trimethoprim were high correlated to pH with -0.994 and -0.927, COD with -0.988 and -0.894, BOD with 0.984 and 0.886 of Pearson's values.

3. Sediment samples

The correlation between antibiotics and sediment samples of effluent characteristic parameters from typical and commercial swine farm (Table 23) showed that the concentrations of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim were high correlated to TOC with 0.941, 0.997, 1.000, -0.997, 0.988, 0.980 and -0.955 of Pearson's values, respectively.

Table 21 Correlation between antibiotics and aqueous of wastewater characteristic parameters from the two swine farms

Water characteristic parameters	LIN		SMR		SM		SMZ		ETM		TMP	
	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig
pH	0.992**	0.000	0.993**	0.000	-0.985**	0.000	-0.992**	0.000	-0.992**	0.000	-0.993**	0.000
COD	0.999**	0.000	0.999**	0.000	-0.998**	0.000	-0.999**	0.000	-0.999**	0.000	-0.999**	0.000
BOD	-0.999**	0.000	-0.999**	0.000	0.999**	0.000	0.999**	0.000	0.999**	0.000	0.999**	0.000
TSS	-0.895*	0.016	-0.895*	0.016	0.920**	0.009	0.895*	0.016	0.895*	0.016	0.894*	0.016

Note: ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; COD, Chemical Oxygen Demand; BOD, Biological Oxygen Demand and TSS, Total Suspended Solid; LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; ETM, erythromycin and TMP, trimethoprim

Table 22 Correlation between antibiotics and suspended solid samples of wastewater characteristic parameters from the two swine farms

Water characteristic parameters	LIN		SMR		SM		SMZ		CPX		ETM		TMP	
	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig
pH	0.823	0.087	-0.551	0.449	-0.987**	0.000	-0.994**	0.006	-0.439	0.561	-0.756	0.244	-0.927*	.024
COD	0.789	0.113	-0.601	0.399	-0.991**	0.000	-0.988*	0.012	-0.313	0.687	-0.729	0.271	-0.894*	0.041
BOD	-0.796	0.107	0.591	0.409	0.993**	0.000	0.984*	0.016	0.290	0.710	0.737	0.263	0.886*	0.045
TSS	-0.696	0.192	0.263	0.737	0.926**	0.008	0.794	0.206	-0.169	0.831	0.851	0.149	0.630	0.255

Note: ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; COD, Chemical Oxygen Demand; BOD, Biological Oxygen Demand and TSS, Total Suspended Solid; LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CFX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

Table 23 Correlation between antibiotics and sediment samples of effluent characteristic parameters from the two swine farms

Water characteristic parameters	LIN		SMR		SM		SMZ		CPX		ETM		TMP	
	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig
TOC	0.941**	0.005	0.997**	0.000	1.000**	0.000	-0.997**	0.000	0.988**	0.002	0.980**	0.003	-0.955*	0.011

Note: ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; TOC: Total Organic Carbon; LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CFX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim

Correlation between antibiotic concentrations and soil property parameters

The correlation between antibiotic concentrations and soil properties parameters such as pH, cations exchange capacities (CEC), organic matter (OM), nitrogen (N) phosphorus (P) and potassium (K) were observed in soil samples from the typical and commercial swine farms with Pearson's correlation.

The correlation between antibiotics and agricultural soil property parameters from typical and commercial swine farms (Table 24) showed that the concentrations of sulfamethazine was very high negative correlated to OM, N and P with -0.564, -0.672 and -0.623, respectively. Moreover, the concentrations of trimethoprim was high positive correlated to K with 0.594 of Pearson's values.

Table 24 Correlation between antibiotics and soil property parameters from the two swine farms

Soil property parameters	LIN		SMR		SMZ		CPX		ETM		TMP	
	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig	Pearson value	Sig
pH	0.103	0.703	0.198	0.445	-0.199	0.477	-0.247	0.522	0.138	0.723	0.270	0.372
OM	-0.282	0.290	0.282	0.272	-0.564*	0.028	-0.532	0.141	-0.072	0.854	0.327	0.275
N	-0.404	0.121	0.134	0.607	-0.672**	0.006	-0.556	0.120	-0.025	0.949	0.217	0.477
P	-0.327	0.217	0.128	0.623	-0.623*	0.013	-0.514	0.157	-0.017	0.965	0.178	0.560
K	-0.019	0.948	-0.049	0.869	-0.446	0.110	-0.533	0.140	0.324	0.395	0.594*	0.032

Note: ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; TOC: Total Organic Carbon; LIN, lincomycin; SMR, sulfamerazine; SM, sulfameter; SMZ, sulfamethazine; CFX, ciprofloxacin; ETM, erythromycin and TMP, trimethoprim OM, Organic Matter; N, Nitrogen; P, Phosphorus and K, Potassium

Fate of antibiotics in the effluent from typical and commercial swine farms

The main aims of the oxidation pond from typical swine farm and a biogas system and lagoon from commercial swine farm as wastewater treatment process can be reduced the organic content of effluent including toxic or trace organic compounds, reduce suspended solids, reduce or inactivate pathogenic bacteria and reduce the nutrient loads discharged to receiving surface waters. However, many antibiotics cannot be removed completely in wastewater treatment processes that are often detected in receiving environment in several reported (Lundborg and Tamhankar, 2017).

The Fate of antibiotics in the effluent may be effected by several factors, such as: effluent characteristics, type of biological process (convectional activated sludge, presence/absence of nitrification and denitrification step, type of biological technology, presence and type of advanced treatment and disinfection (Luigi, & Rizzo, 2012). Antibiotics are released to the environment with leaching of swine effluent and waste utilization as fertilizer applying to agricultural field in unchanged parent form and their metabolites. The metabolites of antibiotics can be transformed back to the parent compound with different pathways such as sorption, adsorption and degradation process depend on their physical properties and environment process (Mojica, & Aga, 2011; Behera et al., 2011; Wegst-Uhrich et al., 2014). This study reported the fate of antibiotics in topic as physical properties of antibiotics, sorption and adsorption and degradation, the detail as below:

1. Physicochemical properties of antibiotics studied

Forty-one antibiotics were target compounds of this study, including sulfamerazine, sulfameter, sulfamethazine, sulfacetamide, sulfaguanidine, sulfanilamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamonomethoxine, sulfachloropyridazine, sulfamethoxazole, sulfadimethoxine, sulfadoxine, sulfisoxazole, sulfaquinoxaline, tetracycline, methacycline, lincomycin, erythromycin, clarithromycin, leucomycin, roxithromycin, Oleandomycin, tylosin, ciprofloxacin, marbofloxacin, fleroxacin, norfloxacin, carbadox, ofloxacin, pefloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, trimethoprim, ormetoprim, narasin and monensin but the results showed that lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, ciprofloxacin and trimethoprim were detected in the

samples. The physicochemical properties of the antibiotic studied are shown in Chapter II.

2. Sorption

The distribution of antibiotics between aqueous phase, suspended solids phase, and sediment phase in the effluent depends on natural sorbents (particles, sediments, humic materials and dissolved organic matters) and their sorption coefficients. The distribution coefficient (K_d) is defined as the ratio of antibiotics in a solid phase and aqueous phase. K_d value can vary with sorbent coefficients (log K_d) and organic carbon sorption coefficients (log K_{oc}) are organized together with characteristics of sorbent (Site, 2001). Then antibiotics will adsorb on particles or sediments/sludge produced as K_d and K_{oc} values.

3. Partitioning of antibiotics between aqueous phase and suspended solid phase of the effluent

Typical swine farm, antibiotics sorption onto suspended solids, sorption coefficients reported in Table 25 show the antibiotics adsorb to solid phase. The partitioning of detected antibiotics, sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim were showed with K_d values of 8,437.97, 625.32, 132.90, 16.07 and 5,529.35, respectively, K_{oc} values of sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim were found of 34,203.35, 2,534.75, 538.71, 65.14 and 22,413.24, respectively. This result indicated that sulfamerazine sorption to suspended solid phase of the effluent was greater than trimethoprim, sulfameter, sulfamethazine and erythomycin. Log K_d of sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim of swine effluent from typical swine farm were found of 3.93, 2.80, 2.12, 1.21 and 3.74, respectively. Log K_{oc} of sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim were found of 4.53, 3.40, 2.73, 1.81 and 4.35, respectively (Table 26).

For commercial swine farm, antibiotics sorption onto suspended solids, sorption coefficients reported in Table 26 show the antibiotics adsorb to solid phase. The partitioning of detected antibiotics, lincomycin, sulfamerazine, sulfamethazine and erythomycin were showed with K_d values of 23.52, 201.45, 9,942.73 and 976.48, respectively, K_{oc} values of lincomycin, sulfamerazine, sulfamethazine and erythomycin

were found of 176.19, 1,508.99, 74,477.39 and 7,314.47, respectively. This result indicated that sulfamethazine sorption to suspended solid phase of the effluent was greater than erythomycin, sulfamerazine and lincomycin. Log K_d values of lincomycin, sulfamerazine, sulfamethazine and erythomycin of swine effluent from commercial swine farm were found of 1.37, 2.30, 4.00 and 2.99, respectively. Log K_{oc} values of lincomycin, sulfamerazine, sulfamethazine and erythomycin were found of 2.25, 3.18, 4.87 and 3.86, respectively (Table 26).

Table 25 Partitioning coefficients of antibiotics (K_d and K_{oc}) in aqueous-suspended solid from the effluent

Compounds	Typical farm (L kg^{-1})		Commercial farm (L kg^{-1})	
	Kd	Koc	Kd	Koc
Lincomycin	ND	ND	23.52	176.19
Sulfamerazine	8,437.97	34,203.35	201.45	1,508.99
Sulfameter	625.32	2,534.75	ND	ND
Sulfamethazine	132.90	538.71	9,942.73	74,477.39
Erythomycin	16.07	65.14	976.48	7,314.47
Trimethoprim	5,529.35	22,413.24	ND	ND

Table 26 Partitioning coefficients of antibiotics (Log K_d and Log K_{oc}) in aqueous-suspended solid phase of effluent from the two swine farms

Compounds	Traditional farm (L kg^{-1})		Commercial farm (L kg^{-1})	
	Log K_d	Log K_{oc}	Log K_d	Log K_{oc}
Lincomycin	ND	ND	1.37	2.25
Sulfamerazine	3.93	4.53	2.30	3.18
Sulfameter	2.80	3.40	ND	ND
Sulfamethazine	2.12	2.73	4.00	4.87
Erythomycin	1.21	1.81	2.99	3.86
Trimethoprim	3.74	4.35	ND	ND

Partitioning of antibiotics between aqueous phase and sediment phase of the effluent

Adsorption onto sediments, the K_d and K_{oc} values of effluent (Table 27) showed that partitioning of antibiotics to sediment from oxidation pond of typical swine farm with K_d values of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim at 246.81, 576.43, 58.70, 31.77, 7.40 and 350.13, respectively. K_{oc} values of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim were found at 1,000.43, 2,336.58, 237.96, 128.78, 29.99 and 1,419.25, respectively. This result indicated that sulfamerazine sorption to sediment phase of the effluent was greater than trimethoprim, lincomycin, sulfameter, sulfamethazine and erythomycin.

Log K_d values of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim from typical swine farm were found at 2.39, 2.76, 1.77, 1.50, 0.87 and 2.54, respectively.

Log K_{oc} values of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythomycin and trimethoprim were found at 3.00, 3.37, 2.38, 2.11, 1.48 and 3.15, respectively (Table 28).

For the partitioning of antibiotics to lagoon sediment from commercial swine farm (Table 27) were found the K_d values of lincomycin, sulfamethazine and erythomycin at 16.00, 9,942.73 and 546.52, respectively. K_{oc} values of lincomycin, sulfamethazine and erythomycin were found at 119.85, 74,477.40 and 4,093.82, respectively. This result indicated that sulfamethazine sorption to sediment phase of the effluent was greater than erythomycin and lincomycin.

Log K_d values of lincomycin, sulfamethazine and erythomycin were found at 1.20, 4.00 and 2.74, respectively. Log K_{oc} values of lincomycin, sulfamethazine and erythomycin were found at 2.08, 4.87 and 3.61, respectively (Table 28).

Table 27 Partitioning coefficients of antibiotics (K_d and K_{oc}) in aqueous-sediment phase of effluent from the two swine farms

Compounds	Typical farm (L kg^{-1})		Commercial farm (L kg^{-1})	
	K_d	K_{oc}	K_d	K_{oc}
Lincomycin	246.81	1,000.43	16.00	119.85
Sulfamerazine	576.43	2,336.58	ND	ND
Sulfameter	58.70	237.96	ND	ND
Sulfamethazine	31.77	128.78	9,942.73	74,477.40
Erythomycin	7.40	29.99	546.52	4,093.82
Trimethoprim	350.13	1,419.25	ND	ND

Table 28 Partitioning coefficients of antibiotics (Log K_d and Log K_{oc}) in aqueous-suspended solid phase of effluent from the two swine farms

Compounds	Typical farm (L kg^{-1})		Commercial farm (L kg^{-1})	
	Log K_d	Log K_{oc}	Log K_d	Log K_{oc}
Lincomycin	2.39	3.00	1.20	2.08
Sulfamerazine	2.76	3.37	ND	ND
Sulfameter	1.77	2.38	ND	ND
Sulfamethazine	1.50	2.11	4.00	4.87
Erythomycin	0.87	1.48	2.74	3.61
Trimethoprim	2.54	3.15	ND	ND

Partitioning of antibiotics between aqueous from effluent and agricultural soil samples with swine wastewater

Antibiotics sorption into agricultural soil from typical swine farm and commercial swine farms, sorption coefficients reported in Table 29 show the antibiotics adsorb significantly to the soil samples. The partitioning of detected antibiotics, lincomycin, sulfamerazine, sulfamethazine, erythomycin and trimethoprim were showed with K_d values ranging from 14.72-37.21, 77.88-1,065.25, 2.15-5,207.76, 0.55- 2,627.52 and ND-748.36 respectively, K_{oc} values of lincomycin, sulfamerazine, sulfamethazine, erythomycin and trimethoprim were found ranging from 110.24- 185.80, 583.35-7,979.37, 8.73-39,009.42, 2.21-19,681.83 and ND-3,033.48, respectively. This result indicated that sulfamerazine sorption to agricultural soil was greater than lincomycin, sulfamethazine, erythomycin and trimethoprim, respectively.

Log K_d of lincomycin, sulfamerazine, sulfamethazine, erythomycin and trimethoprim in agricultural soil from typical and commercial swine farms were found ranging from 1.17-1.57, 1.89-2.92, 0.33-3.72, -0.26-3.42 and ND-2.87, respectively. Log K_{oc} of lincomycin, sulfamerazine, sulfamethazine, erythomycin and trimethoprim were found ranging from 2.04-2.27, 2.77-3.90, 0.94-4.59, 0.34-4.29 and ND-3.48, respectively (Table 30).

Table 29 Partitioning coefficients of antibiotics (K_d and K_{oc}) in aqueous-application soil with swine wastewater from the two swine farms

Compounds	Soil samples									
	S1		S2		S3		S4		S5	
	K_d	K_{oc}	K_d	K_{oc}	K_d	K_{oc}	K_d	K_{oc}	K_d	K_{oc}
Lincomycin	37.21	150.85	19.98	149.63	24.80	185.80	19.24	144.16	14.72	110.24
Sulfamerazine	835.29	3,385.85	77.88	583.35	1,065.25	7,979.37	86.23	645.90	121.94	913.38
Sulfamethazine	2.15	8.73	1,049.26	7,859.59	5,207.76	39,009.42	891.15	6,675.26	233.98	1,752.65
Erythomycin	0.55	2.21	44.68	334.71	959.33	7,186.01	2,627.52	19,681.83	132.21	990.30
Trimethoprim	748.36	3,033.48	ND	ND	ND	ND	ND	ND	ND	ND

Note: S1, grass field soil from traditional farm; S2, S3 and S4 sugarcane field soil from commercial farm and S5, banana field soil from commercial farm

Table 30 Partitioning coefficients of antibiotics (Log K_d and Log K_{oc}) in aqueous-application soil with swine wastewater from the two swine farms

Compounds	Soil samples									
	S1		S2		S3		S4		S5	
	Log K_d	Log K_{oc}	Log K_d	Log K_{oc}	Log K_d	Log K_{oc}	Log K_d	Log K_{oc}	Log K_d	Log K_{oc}
Lincomycin	1.57	2.18	1.30	2.18	1.39	2.27	1.28	2.16	1.17	2.04
Sulfamerazine	2.92	3.53	1.89	2.77	3.03	3.90	1.94	2.81	2.09	2.96
Sulfamethazine	0.33	0.94	3.02	3.90	3.72	4.59	2.95	3.82	2.37	3.24
Erythomycin	-0.26	0.34	1.65	2.52	2.98	3.86	3.42	4.29	2.12	3.00
Trimethoprim	2.87	3.48	ND	ND	ND	ND	ND	ND	ND	ND

Note: S1, grass field soil from traditional farm; S2, S3 and S4 sugarcane field soil from commercial farm and S5, banana field soil from commercial farm

Partitioning of antibiotics

The soil and groundwater nearby the swine farms receives antibiotics from wastewater and manure as fertilization. Moreover they could distributed in various phase such as aqueous, suspended solids, sludge, sediment from flush water, wastewater and biogas system. The result in this study found that the concentrations of lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythomycin-H_2O and trimethoprim in aqueous and suspended solids in flush water samples, aqueous, suspended solids and sludge or sediments in wastewater samples, aqueous and suspended solids in groundwater samples, soil samples and fresh and dried feces samples as showed in Table 31-34.

Table 31 Partitioning of antibiotics in flush water from typical and commercial swine farms

Antibiotics	Flush water			
	Aqueous (ng L^{-1})		Suspended solids (µg kg^{-1})	
	Typical farm	Commercial farm	Typical farm	Commercial farm
Lincomycin	74.22	351.24	62,918.29	9,395.90
Sulfamerazine	4.42	0.92	5,556.01	788.32
Sulfameter	51.03	ND	ND	ND
Sulfamethazine	21.90	598.34	9,296.18	865.03
Ciprofloxacin	ND	ND	17,472.79	3,334.30
Erythromycin	54.94	64.25	3,602.91	5,452.01
Trimethoprim	2.44	286.34	4,620.62	1,061.89
Total	208.95	1,301.09	10,3466.80	20,897.45
Average	34.83	260.22	17,244.47	3,482.91

Table 32 Partitioning of antibiotics in wastewater from typical and commercial swine farms

Antibiotics	Wastewater						
	Aqueous ($ng\ L^{-1}$)		Suspended solids ($\mu g\ kg^{-1}$)		Sediment ($\mu g\ kg^{-1}$)		Sludge ($\mu g\ kg^{-1}$)
	Typical farm	Commercial farm	Typical farm	Commercial farm	Typical farm	Commercial farm	commercial farm
Lincomycin	120.03	734.46	ND	17,275.33	29,624.04	11,751.66	46,659.53
Sulfamerazine	1.79	7.26	7,594.17	1,462.53	518.79	ND	ND
Sulfameter	51.13	ND	31,972.81	ND	3,001.58	ND	ND
Sulfamethazine	773.12	3.72	102,747.26	36,986.96	24,562.79	595.48	38,453.13
Ciprofloxacin	ND	ND	24,553.76	ND	14,641.29	2,350.70	4,219.05
Erythromycin- H_2O	9,614.56	3.07	154,500.08	2,997.80	71,123.61	1,677.83	9,647.60
Trimethoprim	1.47	ND	8,128.14	1,540.20	514.69	634.66	467.75
Total	10,562.10	748.51	329,496.22	60,262.82	143,986.79	53,401.81	99,447.06
Average	1,760.35	187.13	54,916.04	12,052.56	20,569.54	10,680.36	19,889.41

Table 33 Partitioning of antibiotics in fresh feces, dried feces or dried sludge samples from typical and commercial swine farms

Antibiotics	Fresh feces ($\mu g\ kg^{-1}$)		Dried feces ($\mu g\ kg^{-1}$)	Dried sludge ($\mu g\ kg^{-1}$)
	Typical farm	Commercial farm	Typical farm	Commercial farm
Lincomycin	40,229.15	22,524.32	26,614.38	4,090.42
Sulfamerazine	3,158.36	3,242.96	5,858.58	1,987.7
Sulfamethazine	11,803.98	2,349.33	7,658.73	2,292.66
Ciprofloxacin	ND	11,575.57	ND	14,353.39
Erythomycin	24,594.8	1,328.08	21,911.02	4,522.49
Trimethoprim	4,833.13	1,911.87	6,586.56	1,887.45
Total	84,619.42	42,932.13	68,629.27	29,134.11
Average	16,923.88	7,155.36	13,725.85	4,855.69

Table 34 Partitioning of antibiotics in groundwater and soil samples from traditional and commercial swine farms

Antibiotics	Groundwater				Soil (µg/kg)				
	Aqueous (ng/L)		Suspended solids (µg/kg)						
	Traditional farm	Commercial farm	Traditional farm	Commercial farm	S1	S2	S3	S4	S5
Lincomycin	113	59.96	198,789.47	235,535.60	4,466.82	14,671.65	18,217.49	14,134.49	10,809.47
Sulfamerazine	0.98	46.99	6,780.73	43,275.92	751.76	565.39	7,733.69	626.01	885.26
Sulfameter	175.67	ND	ND	ND	ND	ND	ND	ND	ND
Sulfamethazine	3,060.88	100.45	7,093.44	94,853.61	1,665.75	3,903.23	19,372.86	3,315.07	870.40
Ciprofloxacin	ND	ND	15,250.27	36,706.82	3,593.42	ND	1,560.33	1,927.07	2,961.25
Erythomycin-H_2O	ND	693.6	6,028.59	3,112.59	5,245.68	137.18	2,945.15	8,066.50	405.87
Trimethoprim	0.76	72.11	6,727.57	30,239.23	1,100.09	871.17	85,363.39	493.3	693.86
Total	3351.29	973.11	240,670.07	443,723.77	16,823.52	20,148.62	135,192.91	28,562.44	16,626.11
Average	670.258	194.622	40,111.68	73,953.96	2,803.92	4,029.72	22,532.15	4,760.41	2,771.02

Note: S1, grass field soil from traditional farm; S2, S3 and S4, sugarcane field soils from commercial farm and S5, banana field soil from commercial farm

Antibiotic resistance genes in agricultural soil samples

The *tet*O genes were detected in S1 soil sample from traditional swine farm, S3 and S5 soil sample from commercial swine farm. For *tet*M was detected in S3 soil sample from commercial swine farm. However, *tet*O and *tet*M were not detected in control soil samples in this study (Table 35).

In addition, *tet*O and *tet*M genes were showed in an agarose gels of PCR products as expected genes (Figure 25). The data for the similarity DNA analysis were obtained from sequences contained in the BLAST databases available from NCBI. The result showed *tet*O gene sequence were found 100% similar to *Streptococcus suis* BM407 and YM12 strains. For *tet*M gene sequence were found 100% similar to *Streptococcus salivarius* FDAARGOS, *S. agalactiae* C001, Sag158, H002, 2603V/R strains, *S.* phage IPP61, *S. parauberis* NUF1049, *S. epidermidis* pSWS47 (plasmid), *S. constellatus* C1050, *S. pneumoniae* ICE (transposon), *Staphylococcus rostri* tn916 (transposon), RST11, *Staphylococcus pseudintermedius* HKU10-03, *Mycoplasma mycoides* YCp235-1 and GM12, *Aerococcus christensenii* CCUG28831, *Clostridium difficile* ORF1 and Tn916-like transposon, *Escherichia coli* HS13-1, 5Y, M160133, EC1515, EC974, CY4, pSJ_255, *E. coli* pTW4, p41-3 DNA, p15 DNA (plasmid), *Enterococcus faecalis* DENG1, *E. faecalis* pCF10 (plasmid) as showed in Table 36.

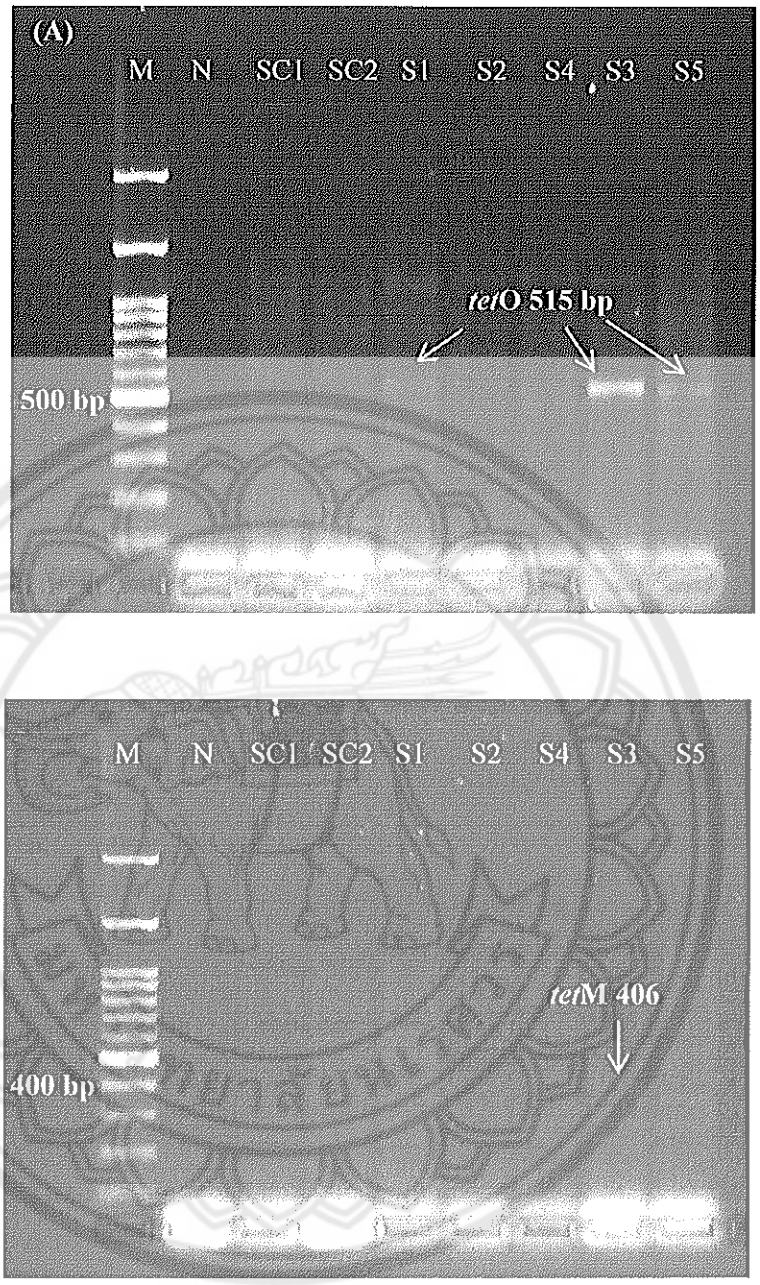


Figure 25 Agarose gels of PCR products stained with ethidium bromide for (A) *tet*O and (B) *tet*M. (A) and (B) Lanes: M, 100 bp DNA ladder; N, negative control; SC1, soil control from traditional farm; SC2, soil control from commercial farm; S1, grass field soil from traditional farm; S2, S3 and S4, sugarcane field soils from commercial farm and S5, banana field soil from commercial farm

Table 35 The detection of ARGs in agricultural soil samples from traditional and commercial swine farm by PCR

Sample	Presence of gene					
	*tet*M	*tet*O	*erm*A	*erm*B	*qnr*A	*qnr*B
SC1	-	-	-	-	-	-
SC2	-	-	-	-	-	-
S1	-	+	-	-	-	-
S2	-	-	-	-	-	-
S3	+	+	-	-	-	-
S4	-	-	-	-	-	-
S5	-	+	-	-	-	-

Note: SC1, soil control from traditional swine farm; SC2, soil control from commercial swine; S1, grass field soil from traditional farm; S2, S3 and S4, sugarcane field soils from commercial farm and S5, banana field soil from commercial farm

Table 36 *tet*O and *tet*M genes sequence similarity (100%)

Genes	Strain name
*tet*O	*Streptococcus suis* BM407
	Streptococcus suis YM12
*tet*M	*Streptococcus salivarius* FDAARGOS
	Streptococcus agalactiae C001
	Streptococcus agalactiae Sag158
	Streptococcus agalactiae H002
	Streptococcus agalactiae 2603V/R
	Streptococcus phage IPP61
	Streptococcus parauberis NUF1049
	Streptococcus epidermidis pSWS47

Table 36 (cont.)

Genes	Strain name
	Streptococcus constellatus C1050
	Streptococcus pneumoniae ICE (transposon)
	Staphylococcus rostri tn916 (transposon)
	Staphylococcus rostri RST11
	Staphylococcus pseudintermedius HKU10-03
	Mycoplasma mycoides YCp235-1
	Mycoplasma mycoides GM12
	Aerococcus christensenii CCUG28831
	Clostridium difficile ORF1
	Clostridium difficile Tn916 (transposon)
	Escherichia coli HS13-1
	Escherichia coli 5Y
	Escherichia coli M160133
	Escherichia coli EC1515
	Escherichia coli EC974
	Escherichia coli CY4
	Escherichia coli pSJ_255
	Escherichia coli pTW4
	Escherichia coli p41-3 DNA
	Escherichia coli p15 DNA
	Enterococcus faecalis DENG1
	Enterococcus faecalis pCF10

CHAPTER V

CONCLUSION AND DISCUSSION

Conclusion

The study of occurrence of selected antibiotics from traditional and commercial swine farms revealed that lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in swine feeds, flush water, wastewater, groundwater, fresh feces, dried feces and agricultural soil, except sulfameter that not found in all samples from commercial farm. In addition, *tet*O and *tet*M genes were found in agricultural soil near the two swine farms. The present study also indicated that antibiotic from swine farms could enter the environment with direct leaching of swine wastewater and waste utilization as fertilizer applying to agricultural field. As a result of different farm managements, especially wastewater treatment process, antibiotic concentrations in the samples from traditional farm were higher than those from commercial farm. Consequently, to reduce contamination of antibiotics from swine farm to the environment should be paid attention.

Discussion

1. Occurrence of antibiotics in swine feed samples

In this study, lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in feeds from traditional and commercial swine farms, except sulfameter that not found in samples from commercial farm. Based on interview with farmers, these antibiotics were commonly used in feed for growth promotion and disease prevention. In fact, all the antibiotics were detected in feed which were mixed on the typical farm by farmer under the experience and decision. For commercial farm, antibiotics were used and mixed in feed on the farm under the control and supervision of farm veterinarians that were conducted on Good Agricultural Practices for pig farm in Thailand. Many antibiotics are not completely absorbed in the gut, resulting in the excretion of the parent

compound and its breakdown metabolites (Boxall et al., 2004). Most antibiotics concentrations in feed samples from typical farm were higher than those from commercial farm. These were due to pigs in typical farm found in different growth stages of swine, including piglets, growing and finishing, and sows; especially, newly weaned piglets, were often fed with various antibiotics with high dosage to prevent and treat diseases.

Lincomycin was found at highest concentrations in feed samples from the two farms. It is commonly used for growth promotion enhanced pig productivity (Pollman et al., 1980) as well as disease treatment and control (Rajić et al., 2006). It is effective in reducing the *Clostridium* spp. infection (diarrheal disease) in all ages of pigs (Silva et al., 2015). Besides, the other antibiotics, including sulfonamides groups, ciprofloxacin, trimethoprim and erythomycin were found in feed samples that are often detected in swine feeds as Zhao et al. (2013; Chen et al., 2012) reported. Furthermore, FDA (2015) reported lincomycin, sulfamerazine, sulfamethazine and erythromycin are approved for use in food-producing animals.

2. Occurrence of antibiotics in fresh feces and flush water samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in aqueous and suspended solids samples of flush water, from the two farms, except sulfameter in commercial farm, which were reflected dosage and frequency of antibiotics in swine feeds in farm samples. Most antibiotics concentrations in aqueous samples from commercial farm were higher those from traditional farm. All the antibiotics were detected in fresh feces and flush water which were reflected the dosage and frequency of antibiotics used in farms. These data demonstrated that swine farms are considered as an important pollution source of various antibiotics to the receiving environments (Qiao et al., 2012). Most antibiotics concentrations in aqueous phase of flush water from commercial farm were higher those from typical farm, while antibiotics concentrations in SS from typical farm were higher than those from commercial farm. This may due to pigs in commercial farm were found older age and more number of pigs than typical farm. Therefore, pigs in commercial farm consume and excrete more than typical farm. Thus, the antibiotics and their metabolites were excreted via feces and urine and contaminated in flush water. Animals consume antibiotics as much as 30 to 90% that

is released into the manure and urine (Sarmah et al., 2006). Moreover, typical farm was operated with open system; the floor was easy to be dirty from slurry, dust and soil around the swine houses and it was not separated between dry and wet area. Thus, the swine houses were flushed with water supply that was contaminated with high antibiotics which may cause of antibiotic increasing in the flush water.

3. Occurrence of antibiotics in dried feces and dried sludge samples

Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in dried feces from typical farm. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in dried sludge which was treated by a biogas system. The concentrations of lincomycin, sulfamethazine and erythromycin were lower in dried feces than in fresh feces. Lincomycin, sulfamerazine, sulfamethazine and trimethoprim were also lower in dried sludge than in fresh feces. Sulfamethazine was found in dried feces and dried sludge reported by Zhang et al., 2015. These suggest that these antibiotics might be degraded or evaporated during the drying process under sunlight and biogas system. Thus, the drying process may be a better way to degrade excessive antibiotics in feces.

4. Occurrence of antibiotics in the effluent samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, and trimethoprim were found in aqueous phase of effluent from typical farm. Sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, and erythromycin were found in aqueous phase of effluent. Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in SS. In addition, lincomycin, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in lagoon sediment. Most antibiotic concentrations in wastewater from typical farm were higher than those from commercial farm. These results suggest that different antibiotic removal efficiencies from wastewater depend on wastewater treatment process corresponding to Gulkowska et al., 2008. The results from the present study demonstrated that sulfamerazine, trimethoprim in aqueous and lincomycin in SS were decreased from flush water by an oxidation pond. In addition, sulfamethazine, erythromycin, trimethoprim in aqueous, ciprofloxacin and erythromycin in SS were decreased from flush water by a biogas system.

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in sediment from oxidation pond. Erythromycin was found at highest concentrations and trimethoprim was found at lowest concentrations in sediment samples from typical farm. For commercial farm, lincomycin, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in lagoon sediment. Lincomycin was found at highest concentrations and sulfamethazine was found at lowest concentrations. Most antibiotic concentrations in aqueous phase, SS and sediment from typical farm were higher than those from commercial farm. These indicated that antibiotic concentrations in wastewater from commercial farm were decreased by the biogas system corresponding to Zhao et al. (2013). These may depend on wastewater treatment methods. In fact, swine wastewater from typical farm was stored in an oxidation pond and drained onto agricultural field, while wastewater from commercial farm was already treated with a biogas system before it was drained onto agricultural field. Thus, antibiotic in the effluent of typical farm were higher than those in commercial farm. However, the antibiotics could not be treated by these wastewater treatment methods. In addition, most antibiotic concentrations in wastewater from the two farms were found in SS higher than sediments and aqueous phase. These suggest that most antibiotics were transferred into the solid phase via sorption as well as eliminated from liquid phase by photodegradation. Such high concentrations in SS would have negative impacts on soil if wastewater and sludge are applied on agricultural field such as effects on soil microbial diversity (Chander et al., 2005). Thus, sorption of antibiotics in solid phase can reduce their mobility, reactivity, and bioavailability for microbial degradation (Hatzinger, & Alexander, 1997).

5. Occurrence of antibiotics in the water supply samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, and trimethoprim were found in aqueous phase of water supply from typical farm. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of water supply from typical farm. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in aqueous phase of water supply. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of water supply.

These antibiotics were found in water supply corresponding with Zhao et al. (2013) and Yao et al. (2017). Base on the farms survey and interview with the farmers, water from the shallow wells farms was pumped and kept in the storage tanks in each farm as water supplies. Water supplies were used for watering pigs and flush manure from swine houses. From this study, antibiotics were found in the effluent samples which were drained on soil in these farms. These suggest that antibiotics might be reach the shallow wells by different pathways (Carvalho, & Santos, 2016). The contamination of antibiotics in the subsoil depends on the frequency of wastewater discharge, physicochemical properties and processes of each compound such as solubility, sorption, degradation as well as soil properties (Boy-Roura et al., 2018).

6. Antibiotics in the agricultural soil samples

Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in grass field soil from typical farm. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in sugarcane field soil. In addition, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in banana field soil. Most antibiotic concentrations in sugarcane field soil were higher than those the other soil samples. The present study also showed that the soil nearby swine farms was contaminated with various antibiotics. Ciprofloxacin and erythomycin were found at high concentrations in grass soil of the typical farm which directly received the effluent from oxidation pond. On the other hand, lincomycin, sulfamerazine, and sulfamethazine were found at high concentrations in agricultural soil from commercial farm. The antibiotic residue in soils was reported in many studies (Boxall, 2004; Hamscher et al., 2005; Martinez-Carballo et al., 2007). Ciprofloxacin, sulfonamides, and tetracyclines could persist in soils a long time (Zuccato et al., 2000), and only a moderate degradation of various tetracyclines occurred within 180 days (Hamscher et al., 2002), while soil without antibiotics used find them due to a habitat of indigenous antibiotics produced by soil microorganisms (Gottlieb, 1976). Thus, soil nearby the swine farms risked for antibiotics accumulated higher than soil without waste from swine farms. However, the occurrence of veterinary antibiotics in the environment matrices from the swine farms depend on breeding, pig age, farm size and farm management.

7. Correlation between antibiotic concentrations and wastewater characteristic

The correlation between antibiotic concentrations and aqueous of flush water characteristic parameters from traditional found that lincomycin was strong negative correlated to TSS when it was tested with Pearson's correlation. For commercial swine farm, lincomycin was strong positive correlated to pH, COD, BOD and TSS, sulfameter was high positive correlated to BOD and trimethoprim was high positive correlated to pH of Pearson's values when they were tested with Pearson's correlation. For suspended solids, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were high negative correlated to pH. Sulfamerazine, sulfamethazine and trimethoprim were high negative correlated to COD. Ciprofloxacin and erythromycin were high negative correlated to BOD and TSS. Ciprofloxacin and erythromycin were high positive correlated to COD. Sulfamerazine, sulfamethazine and trimethoprim were high positive correlated to BOD and TSS. In commercial swine, Lincomycin, sulfamerazine, sulfamethazine and ciprofloxacin were high positive correlated to flush water quality parameters.

For the wastewater, the correlation between antibiotic concentrations and aqueous of wastewater characteristic parameters from oxidation pond (traditional farm) found that only lincomycin high positive correlated to BOD. Lagoon wastewater from commercial swine farm, lincomycin and sulfamerazine were high negative correlated to pH only. In suspended solids, sulfamerazine and ciprofloxacin were high positive correlated to pH, BOD and TSS and these compounds were high negative correlated to COD. Sulfamethazine and erythromycin were high negative correlated to pH, BOD and TSS. For commercial swine, lincomycin was high positive correlated to pH and TSS. Sulfamerazine, sulfamethazine, erythromycin and trimethoprim were high negative correlated to pH and BOD while they were high positive correlated to COD and TSS. In addition, the correlation between antibiotics and oxidation pond or lagoon sediment of wastewater characteristic parameters found that the concentrations of lincomycin, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were high negative correlated to TOC in commercial swine farm while traditional swine farm were not correlated.

For soil samples, the correlation between antibiotics concentrations and soil property parameters from traditional swine farm found that lincomycin, sulfamerazine, sulfamethazine and erythromycin-H_2O were high negative correlated to K. Sulfamethazine was high negative correlated to pH, OM, N and high positive correlate to P. Ciprofloxacin was high positive correlated to pH, OM, N, K and high negative correlated to P. Trimethoprim was high positive correlated to OM, N and K. In commercial swine farm, sulfamethazine was high positive correlated to OM and erythromycin was high positive correlated to N.

In general, the potential degradation and removal efficiencies of antibiotics in wastewater and soil depends on their physicochemical properties and the process of wastewater treatment (Gulkowska et al., 2008). Generally, antibiotic residue are slowly degraded in the wastewater under normal operating conditions of the treatment plants (Abbassi et al., 2016). Thus, these process was one of the great contributing factors to antibiotic concentration in the environment (Rizzo et al., 2013). For the biological wastewater treatment process had not been designed to remove the antibiotics. Especially, swine wastewater was not easily treated with this (Park and Choung, 2007). In various studies were reported that the antibiotic removal from the wastewater treatment plants with different rates (Shokoohi et al., 2017) during 0% (Zuccato et al., 2010), and up to 80% (Li et al., 2011). For the concentration of antibiotics in surface soil were degraded with the abiotic and biotic processes such as hydrolysis and photodegradation (Pikkemaat et al., 2016). However, rate of degradation depends on the type of antibiotics, chemical soil properties, and soil management (Jayanta et al., 2017).

8. Fate of antibiotics in wastewater

The overuse of antibiotics is the primary cause of high concentrations in the slurry sent to wastewater treatment plants. A second factor is the quantity of water and methods used to clean out pig houses (Lallai et al., 2002). Although, the wastewater treatment plants could be remove the suspended solids, nutrients, organic matter, and some pathogens. However, the wastewater treatment plants were not designed for the removal of antibiotics (Pruden et al., 2013). Because of antibiotic compounds are not fully degraded during treatment with the anaerobic digestion and biological processes (Feng et al., 2017 and Shokoohi et al., 2017). Therefore,

antibiotics could be released into the environment via wastewater discharges. In the swine wastewater, the residence time of antibiotics and their distribution between aqueous phase, suspended solid phase and sediment phase from an oxidation pond and a lagoon depends on their physicochemical properties and treatment conditions (Luo et al., 2014) or their capacity to bind to suspended solids and adsorb on sediments.

Antibiotics could be remove during wastewater treatment processes with the adsorption, degradation, disinfection as well as membrane separation (Zhang, 2016). The removal refers to the parent compounds were loss with the degradation process and transformation mechanisms, except their sorption to sludge (Zhou et al. 2013). In addition, the removal of antibiotics in aqueous refers to the parent compounds were loss from aqueous phase during treatment processes (Zhou et al. 2013). However, these parent compounds could be accumulate in suspended solids or sludge phases.

Antibiotics were investigated in the wastewater in this study include lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, ciprofloxacin and trimethoprim, the water solubility of these compounds were showed of 927, 202, 730, 1,500, 1.44, 30,000 and 400 mg/L, respectively (The range of the water solubility is >200 mg/L for high, 5-200 mg/L for mediate and < 5 mg/L for low solubility), indicated that they are a very soluble and they have low tendencies to sorp to the solid phases, except erythromycin. This probably antibiotics concentrations were removed from aqueous phase with the degradation process (Zhou et al, 2013). For erythromycin has solubility of 1.44 mg/L, indicated that it is very low soluble and high tendency sorption onto the sludge. This probably reduces antibiotics concentration in aqueous phase. In addition, many researches were reported that the adsorption process was an important pathway for antibiotic removal from aqueous phase (Kim et al., 2005).

Distribution of antibiotics between aqueous and sludge from a biogas system in this study, K_d values of antibiotics indicating sulfamethazine and erythromycin were adsorbed by sludge phase at 4.01 and 3.50 of log K_d, those with a high log K_d (The log K_d values for low around < 2.6 and high around > 3.6 (Berthod, 2014)). From this study, although sulfamethazine is good binding capacity for water as shown in previous result at the same time, it could good adsorbed by sludge phase. This probably sulfamethazine could be transform with an anaerobic sludge digestion

(Gobel et al., 2005). In this case, precautions may be required for the disposal of sludge to agricultural land, as there are potential risks associated with leaching of desorbed chemicals and the movement of these chemicals into agricultural area (Berthod et al., 2014). For, lincomycin was low adsorbed by sludge phase at 1.80 of log K_d, will remain mainly in the aqueous wastewater.

Distribution of antibiotics between aqueous and suspended solids phases from an oxidation pond and lagoon in this study, K_d values of antibiotics indicating most of antibiotics were stronger sorption from traditional than commercial swine farm. This probably due to result in several factors such as organic carbon and treatment process (Grady et al., 1999). In traditional swine farm, log K_d of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin and trimethoprim were ND, 3.93, 2.80, 2.12, 1.21 and 3.74, respectively. Log K_{oc} of sulfamerazine, sulfameter, sulfamethazine, erythromycin and trimethoprim were ND, 4.53, 3.40, 2.73, ND, 1.81 and 4.35, respectively. For commercial swine farm, log K_d of lincomycin, sulfamerazine, sulfamethazine, erythromycin and trimethoprim were 1.37, 2.30, 4.00, 3.00 and ND, respectively. Log K_{oc} of lincomycin, sulfamerazine, sulfamethazine, erythromycin and trimethoprim were 2.25, 3.18, 4.87, 3.86 and ND, respectively. This suggested that these compounds tendencies to bind to suspended solids and lincomycin, sulfamethazine and erythromycin were good sorption in sludge of biogas from commercial swine farm while, sulfamerazine was strong sorption in suspended solids phase of wastewater from traditional swine farm.

Distribution of antibiotics between aqueous and sediment phase in this study, K_d values of antibiotics indicating lincomycin, sulfamerazine, sulfameter and trimethoprim were strong sorption in traditional swine farm, while sulfamethazine and erythromycin were strong sorption in commercial swine farm. This probably due to in traditional swine farm these was high organic carbon in sediment that is an important factor for sorption. In the traditional swine farm, log K_d of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin and trimethoprim were 2.39, 2.76, 1.77, 1.50, 0.87 and 2.54, respectively. Log K_{oc} of these were 3.00, 3.37, 2.38, 2.11, 1.48 and 3.15, respectively. For commercial swine farm, log K_d of lincomycin, sulfamethazine and erythromycin were 1.20, 2.20 and 2.74, respectively, while log K_{oc} of these were 2.08, 3.08 and 3.61, respectively. This suggested that these compounds

tendencies to adsorb to sediments. Moreover, lincomycin, sulfamerazine, sulfameter and trimethoprim adsorptions in traditional swine farm were stronger than those in commercial swine farm. This probably due to biogas system in commercial swine farm was adsorbed the contaminants during the treatment process (Grady et al., 1999).

However, antibiotic residues are still present in treated swine wastewater in various phases (aqueous, suspended and sediment), indicating that the various matrices of swine wastewater may not be ignored.

9. Fate of antibiotics in agricultural soil

Distribution of antibiotics between aqueous and soil particles in this study, log K_d of lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, ciprofloxacin and trimethoprim were 1.57, 2.92, ND, 0.33, -0.26, ND and 2.87, respectively, while log K_{oc} of these were 2.18, 3.53, ND, 0.94, 0.34, ND and 3.48, respectively from traditional swine farm. For commercial swine farm, log K_d of lincomycin, sulfamerazine, sulfamethazine, erythromycin, ciprofloxacin and trimethoprim were ranging from 1.17 to 1.39, 1.89 to 3.03, 2.37 to 3.72, 1.65 to 3.42, ND and ND, respectively, while log K_{oc} of these were ranging from 2.04 to 2.27, 2.77 to 3.90, 3.24 to 4.59, 2.52 to 4.29, ND and ND, respectively. From the result found that most of antibiotics were stronger sorption in soil sample from commercial farm than traditional farm. This probably due to the soil samples from commercial farm were higher organic matter than traditional farm that is an important factor for sorption. This suggested that these compounds tendencies to adsorb to soils.

In addition, soil properties also strongly affect the behaviors and fates of antibiotics in soil, especially to clay minerals (Stevens, 2009). From this study was found the clay mineral of 30% from traditional farm and 55% from commercial farm. This probably the clay mineral in soil samples from commercial farm was higher than those from traditional farm that is an important.

10. Partitioning of antibiotics

Partitioning of antibiotics was considered in the distribution of each compound or classification between aqueous and solid phases (suspended solid, sludge and soil) in the final effluent. Traditional swine farm, the results found that the concentration of lincomycin in sediment was highest, followed by in soil and lastly in aqueous as Zhang et al., 2013. For commercial swine farm, the concentration of

lincomycin in sludge from biogas system was highest, followed by in suspended solid, soil, sediment and lastly in aqueous. These probably its physicochemical properties and properties of adsorbents (Zhang et al., 2013; Sarmah et al., 2006).

Sulfamerazine, sulfameter, and sulfamethazine concentrations in both traditional farm and commercial farm were found in solid phase more than in aqueous phase. These probably due to low water solubility and high K_d values of sulfonamides which showed in previous chapter. In addition, the final effluents could contain high suspended solids or soils usually contain high levels of suspended solids, indicated that the soil could serve as reservoir for sulfanamides that depends on soil properties (OECD, 2000).

Ciprofloxacin concentrations from traditional swine farm was found in suspended solids with the highest concentration followed by in sediment and soil, respectively. For commercial swine farm, these antibiotic was found in soil with the highest concentration followed by in sludge and sediment, respectively as Dolliver et al., 2008; Zhou et al., 2013. These probably due to ciprofloxacin could degrade with photodegradation process in aqueous and sorbs to particulate organic material (Belden et al., 2007). Moreover, the biogas system in commercial farm could reduce ciprofloxacin, adsorbed to sludge during treatment (Olofsson, 2004).

The concentrations of erythromycin in both traditional farm and commercial farm were found in solid phase more than in aqueous phase as Giger et al. (2003; McArdell et al., 2003). In traditional swine farm was found in suspended solids with the highest concentration followed by in sediment, aqueous and lastly in soil. For commercial swine farm, these antibiotic was found in soil with the highest concentration followed by in sludge, suspended solids, sediment, and aqueous phase, respectively. These probably due to erythromycin tendency to adsorb onto suspended solid which associated with organic carbon on suspended particulate (Martínez-Carballo et al., 2007). However, results from the present study demonstrated that erythromycin was removed from wastewater by a biogas system.

The concentrations of trimethoprim in both traditional farm and commercial farm were found in solid phase more than in aqueous phase. These compound was found in suspended solids with highest concentration, while these compound was found at lowest concentration in aqueous phase. These probably due to

trimethoprim tendency to adsorb onto suspended solid which associated with organic carbon on suspended particulate. In addition, trimethoprim was very low K_{ow} values which showed in previous chapter, indicated that it could high remove from wastewater and adsorb onto soils and sludge (Martínez-Carballo et al., 2007).

11. Occurrence of ARGs in agricultural soils

Antibiotics are extensively used to treat disease and prevent bacterial infection, and also as feed additives to promote growth of animals (Sarmah et al., 2006; Kümmerer, 2009). However, most antibiotics are poorly absorbed with animal body (Zhu et al., 2013) and subsequently excreted in the manure and urine (Sarmah et al., 2006; Berendsen et al., 2015). Moreover, antibiotics were not removed completely with wastewater treatment process (Pruden et al., 2013), resulting in the concentrations of antibiotic residues were found in agricultural soil received wastewater.

Soil contamination with antibiotic residues are a one factor in the selection and dissemination of antibiotic resistant bacteria (Chee-Sanford et al., 2009). Bacterial communities in soil can resist to antibiotics residues, where antibiotics lose their effectiveness and ability to control or kill bacteria growth. Resistant bacteria have a greater chance of survival than those that are susceptible via evolved mechanisms for their self-protection (Alonso et al., 2001) while susceptible bacteria are killed or inhibited by antibiotics (Prestinaci et al., 2015). However, antibiotic resistant bacteria has been reported that it is a natural phenomenon and it can happen everywhere, even without antibiotic contamination with the adaptation and development of bacteria to better survive in their environment conditions in order to thrive and multiply (Brooks et al., 2011). Further, bacteria spread their resistance information beyond the initial organisms. Populations in far-flung regions of the world, who have never known or been treated with antibiotics, or been in contact with people who had been treated with antibiotics, were found to have antibiotics resistance. This demonstrates that resistance is a natural part of the genetic makeup of microbial communities (Fymat, 2017). Moreover, that some antibiotic resistant genes has been found significantly correlate with heavy metals (e.g., Cu, Zn, and as with *fex*A, *fex*B, *cfr*, *sul*1, *tet*W, *tet*O and *tet*S). (He et al., 2014).

General resistance mechanisms include alterations of target sites, limited diffusions or impermeabilities, enzymatic modifications, efflux pumps and genetic adaptations (Blair et al., 2015; Gootz, 2005). For the gene transfer mechanisms of soil bacterial are respond genetically by mutating existing genes (vertical gene transfer) (Sharma et al., 2016) which are naturally present in the chromosomes of bacteria, or by acquiring new genes from other strains or species (horizontal gene transfer) (Von Wintersdorff et al., 2016; Sharma et al., 2016) with the mobile genetic elements include phages, plasmids and transposons mediate this transfer. However, in some circumstances the presence of low levels of the antibiotic in the environment can be the key signal that promotes gene transfer. The movement of genes between bacteria, and even between species by horizontal gene transfer can occur with three main mechanisms include conjugation transduction by bacteriophage and transformation, which is dependent on the native competent state of bacteria as well as cells acquiring induced competency (e.g., the presence of calcium, lightning event) (Chee-Sanford et al., 2009). Thus, antibiotic resistance bacteria can accumulate on mobile genetic element which may facilitate spreading of resistance genes between bacteria of different species and environment by horizontal gene transfer (Roberts, 2005).

In this study, *tet*M, *tet*O (tetracycline resistance genes), *erm*A, *erm*B (macrolides-lincosamide-streptogramin (MLS) resistance genes), *qnr*A and *qnr*B (quinolone resistance genes) were investigated. Several studies showed that *tet*M, *tet*O, *erm*A and *erm*B are common resistance genes in the soil application with swine wastewater (Zhu et al., 2013, Auerbach et al., 2007; Chen et al., 2007). In addition, numerous studies such as Robicsek et al., 2006, Forcella et al., 2010; Cummings et al., 2011 have been reported the isolation of *qnr*A and *qnr*B from the environment source and wastewater effluent in many region. The result in this study showed that *tet*M and *tet*O were found while *erm*A, *erm*B, *qnr*A and *qnr*B were not found in grass field soil from traditional swine farm and sugarcane field soil from commercial swine farms.

The result from this study indicated that *tet*O were found in soil samples from both traditional and commercial swine farms. For *tet*M was detected in soil samples from commercial swine farm. However, the result from analytical of tetracyclines in soil samples from agricultural area near the farm were not found. These may due to, from the interview with owner of traditional swine farm,

tetracyclines were occasionally for the individual pigs and they have excellent absorption by pig's body after injection, these may resulted in low concentration of tetracyclines in wastewater and agricultural soil applied with wastewater. For commercial swine farm, tetracyclines have not been used for 2 years, resulting in that were not found in all samples from these farm. However, *tet*M and *tet*O were detected in the soil samples from traditional and commercial swine farm. These probably due to, tetracycline resistance genes are the most common resistance genes in natural soil (Zhu et al., 2013). The soil is a natural reservoir of antibiotic-producing bacteria containing both intrinsic resistance genes and transferable resistance genes (Popowska et al., 2012). Furthermore, sometime tetracycline resistance genes were found in soil at depths of >30 cm. (Chee-Sanford et al., 2009). In general soil bacteria can be mutated, adapted and developed within their species or between species and species under natural conditions and selection stress in the environment by themselves to survive in their environment, finally they became antibiotic resistance bacteria. Moreover, antibiotic resistance bacteria were encoded by resistance genes that may transfer between pathogens and non-pathogens under selection pressure in the environment (Kruse, & Sorum, 1994) through vertical gene transfer (generation) or horizontal gene transfer mechanism (conjugation, transduction and transformation). The tetracycline resistance via three general mechanisms which included ribosomal protection by large cytoplasmic proteins (e.g., *tet*M, *tet*O, *tet*BP, *tet*Q, *tet*S, *tet*W, *tet*T, *otr*A, *tet*32 and *tet*36), energy-dependent efflux pump (e.g., *tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*G, *tet*H, *tet*I, *tet*J and *tet*Y) and enzymatic inactivation (*tet*X) (Jones et al., 2006 and Kobayashi et al., 2007). The *tet*M and *tet*O, the resistance mechanism is protection of the ribosome at plasmid located (Patterson et al., 2007) that is a major target for tetracyclines resistance (Munita, & Arias, 2016). This suggests that the resistance was linked to plasmids, which theoretically have transfer potential (Aminov, 2011, Gootz, 2010, & Hulscher et al., 2010). In addition, the information and result from this study indicated that metals were added to swine feed for growth promotion and disease control and also found in soil samples. These metals may provide a long-term co-selective pressure for tetracycline resistance which can be encoded on plasmids (Falkow, 1975). Moreover, the result from this study indicated that *Streptococcus* phage were found in soil samples. This phage harboured the genes that confer

tetracycline resistance which may be the source of tetracycline resistance genes (*tet*M) in agricultural soil.

For the data of DNA similarity analysis, target genes were obtained from sequences contained in the BLAST databases available from NCBI. The result showed *tet*O and *tet*M genes sequence were found 100% similar to many strains such as Staphylococcus strain, Streptococcus strain, E. coli strain and Streptococcus phage corresponding with Comeau et al., 2007. These suggest that antibiotics from swine farm may have been found to influence on these bacteria community and resulted in host to acquired antibiotic resistance gene in these bacteria. Furthermore, Wang et al., 2018 reported that bacteriophage DNA contained the several ARGs. These may affect to the contribution of bacteriophages to the dissemination of resistance genes in soil via runoff, leaching and fertilization.

In addition, ciprofloxacin resistance genes including *qnr*A and *qnr*B genes and erythromycin and lincomycin resistance genes including *erm*A and *erm*B genes were investigated in this study. However, the result showed that *qnr*A, *qnr*B, *erm*A and *erm*B genes were not found in soil samples collected from agricultural area which were applied with wastewater from swine farms. Although, ciprofloxacin, erythromycin and lincomycin were used in these farms and contaminated in soil samples.

In general, the plasmid carrying *qnr*A and *qnr*B genes provided resistance to quinolone class (Jacoby et al., 2014). Resistance to quinolones is generally caused by two main mechanisms which included alteration of target enzymes caused by chromosomal mutations in encoded genes (*gyr* and *par* genes), leading to decreased affinity for the drug and reduced intercellular accumulation due to increased efflux of the drug (Oh and Edlund, 2003), encoding by *qnr* gene, which blocks the action of quinolones on the DNA gyrase and topoisomerase IV (Fàbrega et al., 2009). Over 76% of the quinolones (e.g., ciprofloxacin) resistance was shown to be mediated by efflux (Walsh, & Duffy, 2013). For the plasmid carrying *erm*A and *erm*B genes provided resistance to erythromycin and lincomycin. Erythromycin and lincomycin are effect to inhibit protein synthesis in Gram-positive and Gram-negative bacteria by binding to either the 30S or 50S subunits (Tenson, & Mankin, 2006). There are three different mechanisms of resistance including, the use of an energy-dependent

efflux encoded by *msr* gene, production of inactivating enzymes encoded by *lun* gene and alteration of 23S rRNA methylases encoded by *erm* genes (Wang et al., 2008).

However, several studies reported that significant increases resistance gene abundances in agricultural soils receiving wastewater from swine production at low antibiotic residues (ng to mg/kg soil) (Li et al., 2012; Marti et al., 2014; Scott et al., 2018). However, *qnr*A, *qnr*B, *erm*A and *erm*B were not found. These may due to the agricultural practices like field burning of crop and tillage. In this study area, sugarcane field were burned between rotations of sugarcane plantation. This may cause to negative effect to soil bacterial community and assisting of antibiotic resistance genes in agricultural soil (Pinheiro et al., 2010, Souza et al., 2012, Rachid et al., 2012 and 2013). Dunivin and Shade, 2018 reported that the soil temperatures increased can reduce soil antibiotic resistance genes in community-level diversity. For traditional swine, the grass field applied with wastewater from this swine farm were tillage once per year after harvest (personal interview). Tillage can effect to soil microbial community (Wang et al., 2012) and substantially reduce the accumulating of antibiotic resistance genes in soil (Dolliver, & Gupta, 2008; Kay et al., 2005).

Recommendation

The results from this research could be benefit to the public, swine farmer, relevant organization and those persons interested. The policy recommendations will lead to the good practices of swine farm owners for both typical and commercial swine farms and those who are manufacturer, supplier and importer antibiotics. The relevant organization should have policies to encourage farmers, raise awareness of the farmer for proper use of antibiotic to improve their understanding and help swine farm owner to produce high quality pig products and safety for consumers and environment. In addition, the relevant organization should determine the laws and guidelines to control the usage of antibiotic according to the veterinary prescription in swine farms or other livestock. Providing the veterinary services, diagnosis, technical support, guidance and farm management, according to the good agricultural practices, should be supported to swine farm especially typical swine farm. Moreover, continuously monitors the antibiotic usage in swine farms should be conducted by the relevant

organization. All these public policies will lead to green and clean environment, safety product and good health of consumer.

Moreover, in order to understanding of contamination of antibiotics from swine farm in environment, further research is needed to done in various aspects including the fate and transportation of antibiotics in environment, occurrence of antibiotic bacteria and gene in environment. Besides, distribution and existing of antibiotic resistant gene in ecosystem should be investigated and risk assessment in both ecological and human health level also be paid attention.

For further work, quantitative real-time-PCR method which was a suitable method for quantifying the copy numbers or analyzing the abundances of ARGs or other genes of interest may be adopted to use for analysis of resistance genes.

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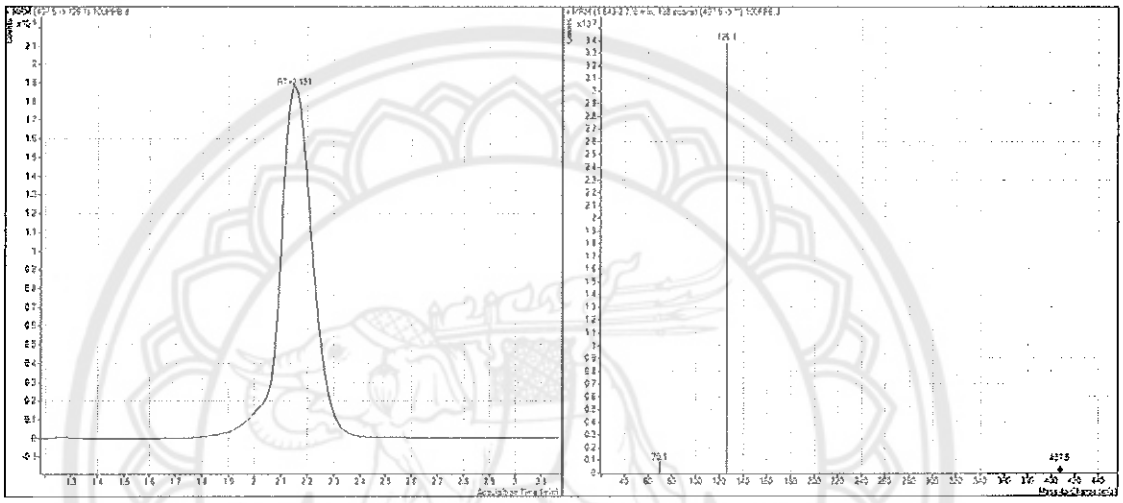
APPENDIX

MRM Chromatogram of RRLC-MS/MS spectrum of selected antibiotics

Lincomycin

R.T. (min) 2.151

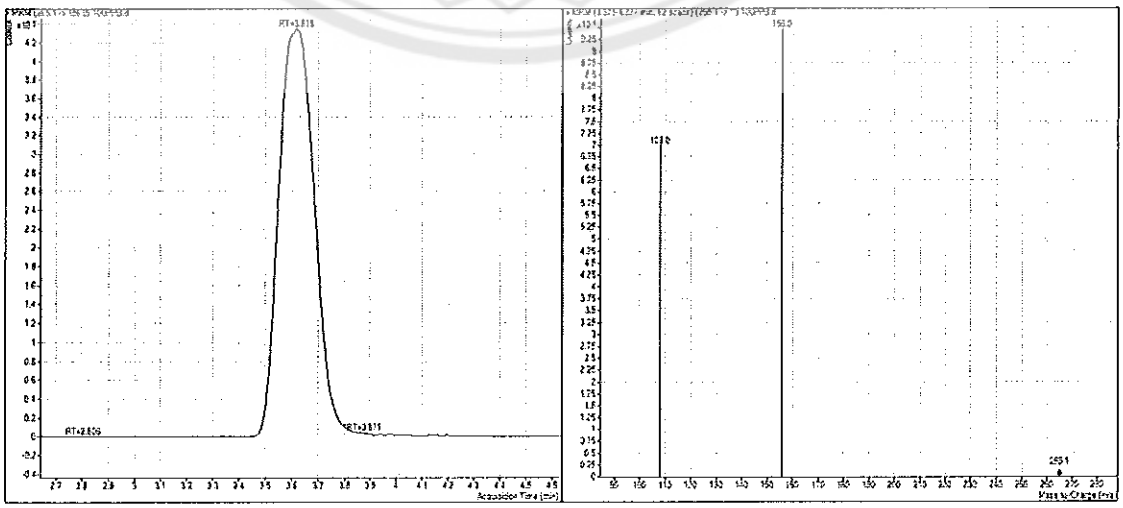
MRM-transitions <u>407.5→126.1</u>
407.5→70.1



Sulfamerazine

R.T. (min) 3.618

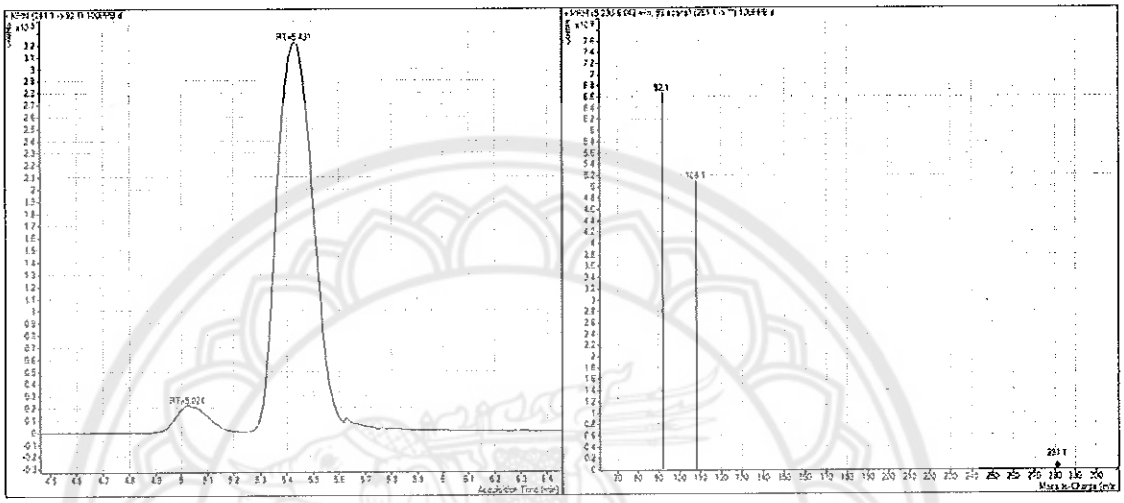
MRM-transitions <u>265.1→156.0</u>
265.1→108.0



Sulfameter

R.T. (min) 5.431

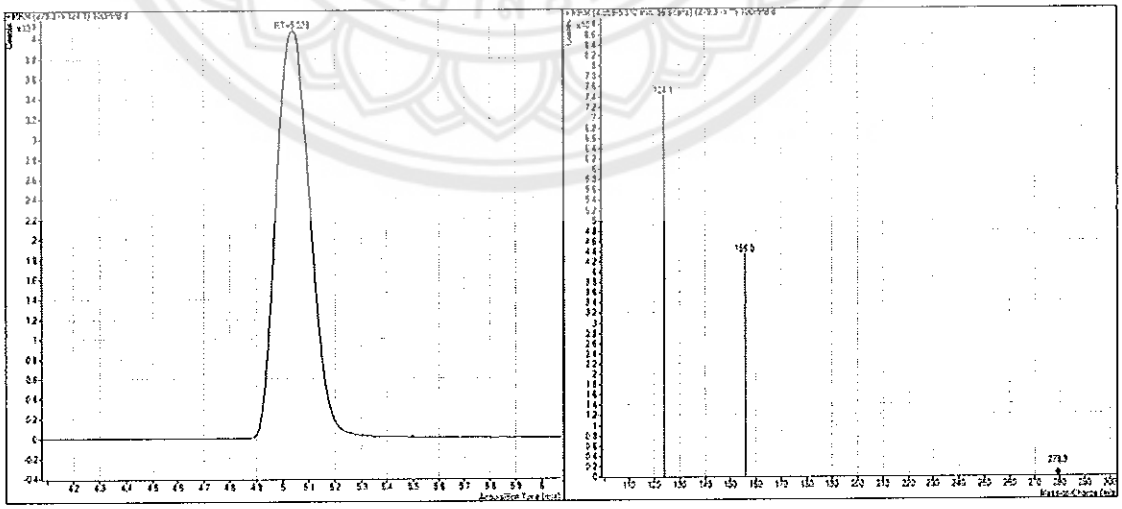
MRM-transitions 281.1→92.1
281.1→108.1



Sulfamethazine

R.T. (min) 5.039

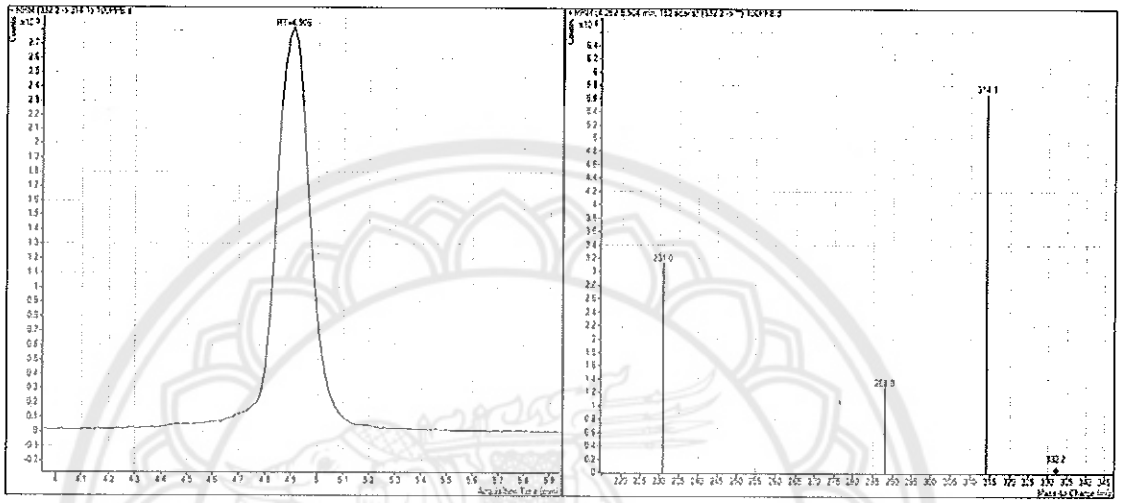
MRM-transitions 279.3→124.1
279.1→156.0



Ciprofloxacin

R.T. (min) 4.906

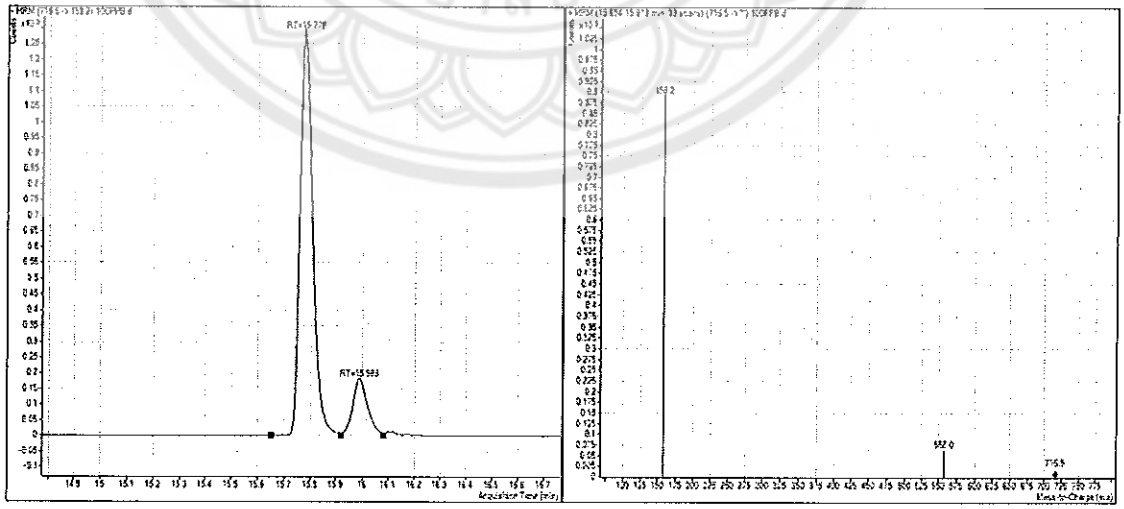
MRM-transitions 332.2→314.1
332.2→288



Erythromycin-H_2O

R.T. (min) 15.778

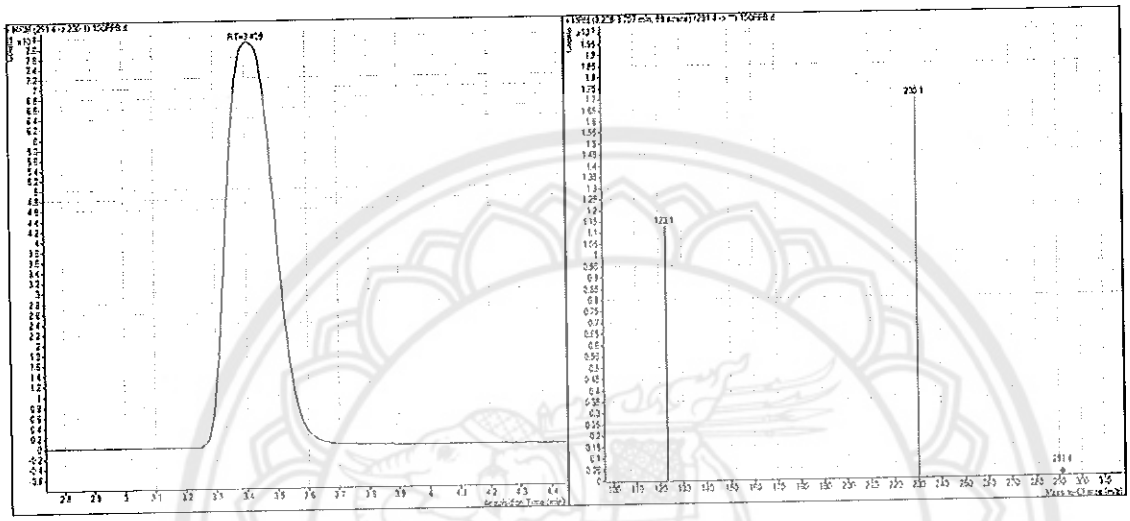
MRM-transitions 716.5→158.2
716.5→558



Trimethoprim

R.T. (min) 3.323

MRM-transitions <u>294.4→123.0</u>
294.4→230.0



Manufacturer's protocol of GenElute™ Soil DNA Isolation Kit product

Reagents to be prepared

Reagents to be prepared following product description of Sigma-Aldrich as show in the index II. Prepare the following before beginning this procedure:

1. Prepare a working concentration of Wash Solution A by adding 42 mL of 96 - 100 % ethanol to the supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

2. This kit is provided with 2 separate columns, humic acid removal columns; column has white contents with a blue plastic o-ring and spin columns; column has white contents with a grey plastic o-ring. Storage/Stability all solutions should be kept tightly sealed and stored at room temperature. The detail of procedure as below.

Procedure for purifying total DNA

Lysate Preparation

1. 250 mg of soil was added to a bead tube, and add 750 µL of lysis buffer G briefly to mix soil and lysis buffer G.

2. Add 100 µL of Lysis additive A, and vortex briefly.

3. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment. Vortex for 5 minutes using a flat-bed vortexer at maximum speed.

4. Centrifuge for 2 minutes, at 14,000 rpm.

5. Transfer up to 450 µL of supernatant into a DNase-free microcentrifuge tube.

6. Add 100 µL of binding buffer I, mix by inverting the tube for a few times, and incubate for 5 minutes on ice.

7. Spin the lysate for 2 minutes at 14,000 rpm to pellet any protein and soil particles.

8. Transfer up to 450 µL of supernatant to a DNase-free microcentrifuge tube using a pipette. Then, OSR solution 50 µL were add and inverting the tube at a few times for mix, after that incubate the tubes for 5 minutes on ice. Spin the lysate for 2 minutes at 14,000 rpm to pellet any protein and soil particles.

9. Transfer up to 450 μL of supernatant into a blue o-ring column using a pipette. Spin the column at 8,000 rpm for 1 minute. Don't discard the flow through that contains DNA.

10. Lastly, 230 μL of 96-100% ethanol were add directly to the flow through.

Binding to Column

1. Using a pipette, gently mix the lysate and ethanol and then apply all of the clarified lysate with ethanol (approximately 630 μL) to the grey o-ring column and centrifuge for 1 minute at 8,000 rpm.

2. Leave the flowthrough and reassemble the spin column using by the collection tube.

Column Wash

1. 500 μL of buffer SK were applied into the column and centrifuge for 1 minute at 8,000 rpm.

2. Leave the flowthrough and reassemble the spin column with its collection tube.

3. 500 μL of wash solution A were applied into the column and centrifuge for 1 minute at 8,000 rpm.

4. Leave the flowthrough and reassemble the spin column with its collection tube.

5. Finally, spin the column for 2 minutes at 14,000 rpm in order to thoroughly dry the resin and leave the collection tube.

DNA Elution

1. Put the column into a fresh 1.7 mL elution tube.

2. 100 μL of elution buffer B were add into the column and incubate for 1 minute at room temperature.

3. Centrifuge for 1 minute at 8,000 rpm.

4. If desired, additional elution may be performed with repeating steps 4.2 and 4.3 using 50 μL of elution buffer in a different elution tube. The total yield can be improved by an additional 20-30% when this second elution is performed.

Storage of DNA

For a few days, the purified genomic DNA can be stored at 4°C while it storage at -20°C is recommended for longer term. The DNA was determined using the spectrophotometer and agarose gel electrophoresis.