

Antimicrobial Use and Multidrug-Resistant *Salmonella* spp., *Escherichia coli*, and *Enterococcus faecalis* in Swine from Northern Thailand

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Abstract

The aim of this study was to isolate antimicrobial-resistant bacteria from intensive commercial and backyard swine operations (farms) in Northern Thailand and to characterize antimicrobial usage among swine farmers. A total of 51 swine farms in Chiang Mai, Lampoon, and Nan provinces were visited from February to October 2011 to collect swine fecal samples for bacterial isolation (*Salmonella* spp., *Escherichia coli*, *Enterococcus faecalis*) and antimicrobial resistance testing. A survey about production intensity, management practices, animal health, and antimicrobial usage was conducted with a sub-set of farms (n = 20) located in Nan province. The farm-level prevalence of drug-resistant *E. coli*, *E. faecalis*, and *Salmonella* spp. was 96, 35, and 18%, respectively. Of the four surveyed commercial operations (median = 58 pigs/farm), 75% routinely administered antimicrobials to pigs (> 50% of animal life). In the 16 backyard farms surveyed (median = 6 pigs/farm), 89% used antimicrobials intermittently (10-50% of animal life) or episodically (< 10% of animal life) and one farm routinely administered antimicrobials to pigs. Herd size was moderately positively correlated ($R^2 = 0.44$) to the number of resistance traits identified on a farm. These data indicate that reservoirs of multidrug resistance are widely present in swine farms of all sizes in Northern Thailand and may be affected by farm size and antimicrobial usage practices on the farm.

Keywords: antimicrobials, *E. coli*, multidrug-resistant bacteria, *Salmonella*, swine

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Introduction

Antimicrobial resistance among animals raised for food is of growing concern in the Asian-Pacific region (Archawakulathep et al., 2014). Antimicrobials are often given to food animals to treat or control disease, or for growth promotion (Silbergeld et al., 2008). In Thailand these drugs can be purchased over the counter, administered by a veterinarian, a veterinarian technician or by a farmer, and may be the same types of antimicrobials used in human medicine. Antimicrobial use in food animals leads to the emergence of antimicrobial-resistant bacteria in the environment, in animal workers, and in foods (Khachatourians, 1998; Gilbert et al., 2012; Keelara et al., 2013; Pletinckx et al., 2013). The use of antimicrobials in food production can lead to development of resistance genes in commensal enteric bacteria and these bacteria may serve as a reservoir for pathogenic bacteria (Phongpaichit et al., 2007; Wright, 2007; Silbergeld et al., 2008). In Thailand, antimicrobial-resistant strains of *Salmonella*, *Escherichia coli* and *Campylobacter* have been isolated from swine (Hanson et al., 2002; Padungtod et al., 2006; Chuanchuen and Padungtod, 2009) and pork has been shown to be a route of transmission for resistant bacteria to humans (Lertworapreecha and Sutthimusik, 2012).

Swine are one of the major food animals produced and consumed in Thailand, with 9.7 million pigs raised by about 227,000 farmers in 2011 (Thailand Ministry of Agriculture, 2011a; 2011b). Farm size and business model vary widely, from backyard swine farms consisting of fewer than 20 pigs to commercial operations that raise hundreds to thousands of animals as contract growers or private businesses (Thailand Ministry of Agriculture, 2011a; 2011b). As some of the authors have observed, swine raised in backyard farms are typically butchered and sold locally in a village or eaten by the farmer or their family, while swine from commercial farms are processed in factories and distributed to larger markets. Swine farms in the East and Central regions of Thailand have the highest number of animals per farm, which is typical of commercial production, while Northern Thailand has pig farms with fewer animals and is more consistent with a mixture of backyard farms and commercial farms (Thailand Ministry of Agriculture, 2011b).

The United Nations Food and Agriculture Organization (UN FAO) acknowledges that there exists a lack of data in the peer reviewed literature on the use of antimicrobials in livestock production in Asia (Otte et al., 2012). This study addresses a critical data gap recognized by the UN FAO. The purpose of this study was to characterize antimicrobial usage in Northern Thailand swine farms of various sizes and to determine the nature of antimicrobial resistance in selected bacteria (*E. coli*, *Enterococcus faecalis*, *Salmonella* spp.) isolated from pig fecal samples in the same region. We selected *E. coli* and *Salmonella* spp. to compare our findings with previous studies of pigs in Thailand, and we selected *E. faecalis* because it is a commensal bacterium that we expected to find in swine feces, yet also can share resistance traits with other bacteria.

Materials and Methods

Ethics Statement: An animal care and use certificate was not required for this research because no animals were involved in the study. Animal waste was collected from farms with permission from farm operators. Farm operator surveys were approved by the Animal Research Expertise Committee, Department of Livestock Development, Thailand.

Study design and farm survey: Fecal samples were collected from a random sample of 15 small swine farms (1-20 pigs), 15 medium swine farms (21-100 pigs) and 15 large swine farms (> 100 pigs) from Nan province for bacterial isolation (*Salmonella* spp., *E. coli*, *E. faecalis*), antimicrobial resistance analysis, and to link these findings to a survey of swine farm operators. The farm operator survey and sampling scheme were described in a previous study (Hinjoy et al., 2013). The survey by Hinjoy and colleagues was employed in this study in Nan Province to capture the intensity of animal production, management practices, animal health, and antimicrobial usage. Due to limited time for sampling, the final number of Nan province farms sampled was n = 21 for swine fecal samples and n = 20 for the survey.

To increase the sample size and meet the desired distribution of small, medium and large farms, the study was expanded and a convenience sample (a non-random sampling method based on the ease of investigators accessing farms) was conducted of farms in Chiang Mai (n = 22) and Lampoon (n = 8) provinces. Chiang Mai and Lampoon provinces were selected because they are in Northern Thailand and near the co-author's (PT) laboratory at Chiang Mai University. Swine farms from all three provinces were visited between February and October 2011 with farm subdistrict locations depicted in Fig 1. Information about swine farm location and herd size was collected at every farm, however, due to budget and personnel constraints, the farm operator survey was only collected in Nan province.

Sample collection: Swine fecal samples (0.3 to 0.5 grams/sample) were collected randomly from six to twelve animals per farm (n = 43 farms), except for farms with fewer than six animals (n = 8 farms) where one to five animals were sampled. Samples were collected by inserting swabs into freshly voided swine feces. Swabs from each farm were pooled in 5 mL 0.1% peptone buffer solution transport media at 4°C and analyzed in a laboratory at Department of Microbiology, Faculty of Medicine, Chiang Mai University.

Salmonella spp.: *Salmonella* was enriched by mixing 1 mL of transport media with 10 mL Rappaport-Vassiliadis broth and incubated overnight at 35°C. The enrichment was streaked on *Salmonella-Shigella* agar and incubated overnight at 35°C. One to five black colonies were selected for biochemical analyses with triple sugar iron (TSI), motility-inodle-lysine (MIL), ornithine decarboxylase, malonate, dulcitol, and urease. *Salmonella* antisera were used to identify suspected organisms. API 20 E strips (BioMerieux)

were used to identify some doubtful organisms. Isolates were stored in 20% glycerol- tryptic soy broth at -80°C.

***E. coli*:** Ten microliters of transport media were spread on MacConkey agar plates incubated overnight at 35°C. One to five pink colonies were collected per sample and biochemically analyzed with TSI, MIL, citrate, methyl red & Voges-Proskauer. API 20 E strips were used to identify some doubtful organisms. Confirmed isolates were stored as described above. We did not attempt to distinguish normal flora from pathogenic *E. coli* in this study.

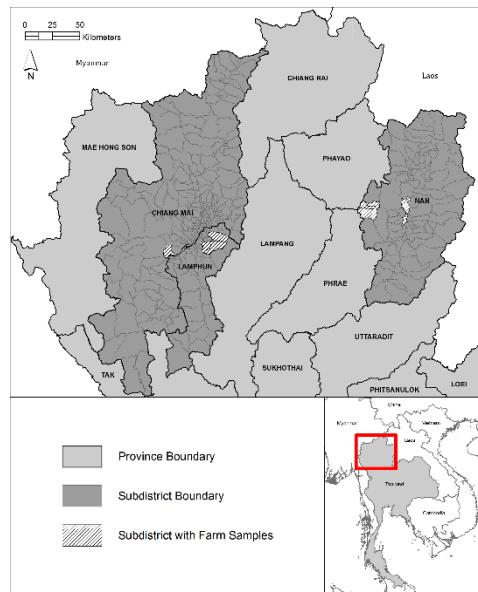


Figure 1 Map of Thailand study sites. Farms were in Chiang Mai ($n = 22$), Lampoon ($n = 8$), and Nan ($n = 21$) provinces. The location of farms was not reported to protect the privacy of the farmers.

***Enterococcus faecalis*:** Ten microliters of transport media were spread on bile esculin (BESC) agar plates, and incubated overnight at 35°C. One to five black colonies were collected per sample and biochemically characterized on BESC agar, for growth in the presence of 6.5% NaCl, and positive results in a pyrrolidonyl arylamidase test. Conventional physiological testing was used to identify some doubtful organisms using methods described previously (Teixeira and Facklam, 2003). Confirmed isolates were stored as described above.

Antimicrobial resistance testing. Antimicrobial resistance testing was performed by a disc diffusion method as described previously (Clinical Laboratory Standards Institute, 2013). The panel of antimicrobials we selected was, in part, based on the antimicrobials used by the US National Antibiotic Resistance Monitoring System (NARMS). Up to three isolates of each bacteria-type per farm were tested for antimicrobial resistance. The upper limit on the number of isolates per farm was intended to prevent farms with larger numbers of isolates from being overrepresented in the dataset. *E. coli* isolates ($n = 126$) and *Salmonella* spp. isolates ($n = 17$) were analyzed for resistance to the following antimicrobials and

concentrations: amoxicillin/clavulanic acid (AMC, 20/10 ug), ampicillin (AMP, 10 ug), cefoxitin (FOX, 30 ug), ceftiofur (EFT, 30 ug), ceftriaxone (CRO, 30 ug), chloramphenicol (C, 30 ug), ciprofloxacin (CIP, 5 ug), gentamicin (CN, 10 ug), kanamycin (K, 30 ug), nalidixic acid (NA, 30 ug), streptomycin (S, 10 ug), tetracycline (TET, 30 ug), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 ug). *E. faecalis* isolates ($n = 29$) were analyzed by disc diffusion for resistance to: chloramphenicol (30 ug), ciprofloxacin (5 ug), clindamycin (DA, 2 ug), erythromycin (E, 15 ug), kanamycin (30 ug), linezolid (LZD, 30 ug), penicillin (P, 10 units), quinupristin/dalfopristin (QD, 15 ug), streptomycin (10 ug), sulfamethoxazole (RL, 300 ug), tetracycline (30 ug), tigecycline (TGC, 15 ug), and vancomycin (V, 30 ug). Antimicrobial susceptibility was recorded as resistant, intermediate or susceptible. Antimicrobial resistance was assessed according to the guidelines of the Clinical and Laboratory Standards Institute (Clinical Laboratory Standards Institute, 2013). Multidrug resistance was defined as resistance to three or more classes of drug.

Extended spectrum beta-lactamase (ESBL) detection: *E. coli* isolates that were resistant to ceftazidime and/or cefotaxime were tested for extended spectrum beta-lactamase (ESBL) by double disc synergy test (Jonathan, 2005) and modified Hodge test (Rand et al., 2011).

Data analysis: Linear regression and R-squared calculations were performed on plots of the \log_{10} number of swine on a farm and the total number of drug-resistance traits. The mean number of drug-resistance traits at farms from different provinces was compared using a Kruskal-Wallis test and a Dunn's multiple comparison post-test because the data were not normally distributed. Differences between mean number of antimicrobial resistance traits were compared for several binary factors [farm type (commercial vs backyard); use of in-house feed; use of store-bought feeds; cleaning frequency (daily vs less than daily); antimicrobial administration frequency (routine vs intermittent or episodic); antimicrobial administration (under veterinarian supervision vs at farmers discretion) using a Student's T-test. Significance for these tests was set at an alpha = 0.05. Descriptive statistics from the survey data were also performed.

Results

Farm size: A total of 51 farms in Chiang Mai, Lampoon and Nan provinces were sampled, consisting of 13 large farms, 19 medium farms, and 18 small farms. In Lampoon province eight large farms were sampled, with a mean size of 899 pigs. In Chiang Mai province, four large farms, 15 medium-sized farms, and three small farms were sampled, with a mean size of 110 pigs. In Nan province, 1 large farm, 4 medium farms, and 15 small farms were sampled, with a mean size of 27 pigs. Farmers in the study in Nan province were administered a survey to ascertain management practices and antimicrobial use at specific farms.

Farm survey: Twenty of 21 study farms in Nan province participated in the survey (Table 1). Based on

the survey responses, the farms in Nan province were categorized as either backyard producers ($n = 16$) or commercial producers ($n = 4$); the latter included private companies and contract growers. The median farm size for backyard farms was six animals (range: 1-20). The median size of commercial farms was 58 animals (range: 30-277). Eighty-one percent of the backyard farms raised swine in open facilities, while no commercial producers used open facilities, and instead used partially-open or closed enclosures. Mixing feed on the farm was found to be common among the backyard farmers (75%), while the commercial growers purchased feed from stores (100%). Four backyard farms (25%) mixed human waste into pig feed. The commercial growers cleaned enclosures and water troughs with a greater frequency (50%, 1 times/d) than the backyard growers (56%, 1 times/week) and were more likely to use disinfectants when cleaning floors (sometimes or always use disinfectants: 100% vs 57%). At both types of operations, manure generated onsite was used near the property, typically as fertilizer for crops on land owned by the operation. Half of the backyard farmers ($n = 8$) did not use dedicated footwear and clothing during farm work, while all commercial farmers used footwear and clothing. Most backyard farmers (69%) used a local veterinarian to supervise antimicrobial use, while the commercial farms provided antimicrobials at their own discretion using their own staff veterinarians. Seventy-five percent of the commercial operations and 11% of the backyard operations reported routinely administering ($> 50\%$ of animal life) antimicrobials to pigs, which might be used for growth promotion, while 56% and 33% of the backyard farmers used antimicrobials intermittently (10-50% of animal life) and episodically (< 10% of animal life), respectively. The commercial producers reported using the antimicrobials enrofloxacin, lincomycin, penicillin, tiamulin, and tyllosin; the backyard producers reported using the antimicrobials amoxicillin, cephalexin, colistin, gentamicin, kanamycin, and penicillin.

Prevalence of antimicrobial-resistant bacteria in swine farms: Antimicrobial-resistant bacteria were detected in 50 of 51 farms in the study (Table 2). Resistant *E. coli* were detected on all farms in Chiang Mai and Lampoon provinces, and 90% of farms in Nan province. Resistant *E. faecalis* were detected on 50% of the farms in Lampoon province, 48% of the farms in Nan province, and on 18% of Chiang Mai farms. Resistant *Salmonella* spp. were detected on 36% of Chiang Mai farms, only on a single farm in Lampoon province, and not on any farms in Nan province. Some farms contained multiple types of resistant bacteria: 6% of the farms contained all three bacteria (resistant *E. coli*, *E. faecalis* and *Salmonella* spp.); 33% had resistant *E. coli* and *E. faecalis*; 18% had resistant *E. coli* and *Salmonella* spp.; and 6% had resistant *E. faecalis* and *Salmonella* spp.

Prevalence of antimicrobial resistance and susceptibility by type of antimicrobial: Resistance to tetracycline and/or beta-lactams occurred in 99% of the *E. coli* isolates, and some level of resistance was detected against all classes of antimicrobials tested (Fig 2A), including cephalosporins, which

were examined in more detail. Among the *Salmonella* spp. isolates, resistance to tetracycline, ampicillin, trimethoprim/sulfamethoxazole, and chloramphenicol was observed (Fig 2B). For the *E. faecalis* isolates, over 70% were resistant to clindamycin, erythromycin, streptomycin, sulfamethoxazole, or tetracycline (Fig 2C).

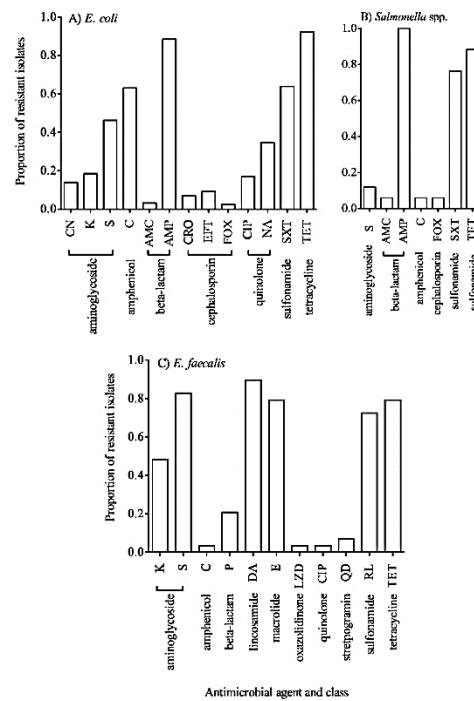


Figure 2 Type and proportion of antimicrobial resistance in A) *Escherichia coli*, B) *Salmonella* spp., and C) *Enterococcus faecalis* isolates from swine feces in three provinces in Thailand. Acronyms for antimicrobials are as follows: amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (C), ciprofloxacin (CIP), gentamicin (CN), ceftriaxone (CRO), clindamycin (DA), erythromycin (E), ceftiofur (EFT), cefotaxime (FOX), kanamycin (K), linezolid (LZD), nalidixic acid (NA), penicillin (P), quinupristin/dalfopristin (QD), sulfamethoxazole (RL), streptomycin (S), trimethoprim/sulfamethoxazole (SXT), tetracycline (TET), tigecycline (TGC), and vancomycin (V).

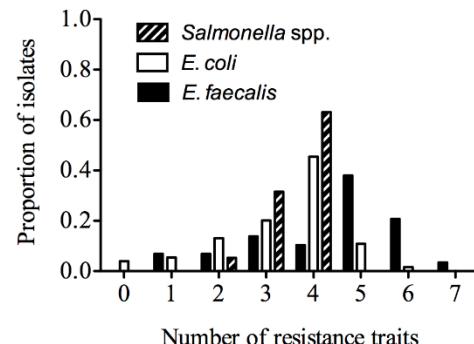


Figure 3 Proportion and number of resistance traits among *Salmonella* spp., *Escherichia coli*, and *Enterococcus faecalis* isolates from swine feces in three provinces in Thailand

Table 1 Survey of farm management practices and antimicrobial use in Nan province ^a

Factors	Number of Farms (percent)	
	Commercial production ^b	Backyard production
N	4	16
Number of pigs (range)	30 to 277	1 to 20
Type of enclosure		
open	0	13 (81)
partially open	2 (50)	3 (19)
closed	2 (50)	0
Feeding Practices		
use compound feeds	0	3 (19)
use industrial and agricultural byproducts	4 (100)	15 (94)
use domestic waste	0	4 (25)
Feed Source		
use in-house feed ^c	1 (25)	12 (75)
bought feed from store	4 (100)	7 (44)
Cleaning of units		
never or < 1 per month	0	3 (19)
once per month	0	0
once per week	2 (50)	11 (69)
once per day	2 (50)	2 (13)
Cleaning of floors with disinfectants		
never	0	7 (44)
sometimes	4 (100)	6 (38)
always	0	3 (19)
Clean water troughs		
never or < 1 per month	1 (25)	5 (31)
once per month	0	0
once per week	1 (25)	9 (56)
once per day	2 (50)	2 (13)
Management of manure		
collected in septic tank	0	1 (6)
used as fertilizer for crops	3 (75)	12 (75)
discarded nearby to premises	4 (100)	13 (81)
Frequency of using dedicated footwear or clothing while farming		
never	0	8 (50)
sometimes	0	3 (19)
always	4 (100)	5 (31)
Animal health		
veterinarian provided to pigs	0	0
antimicrobials provided under supervision of a local veterinarian	0	11 (69)
antimicrobials provided by farmer discretion	4 (100)	5 (31)
Antimicrobials use		
routine (>= 50% lifespan of pig)	3 (75)	1 (11)
intermittent >= 10% - < 50%	0	5 (56)
episodic (< 10%)	1 (25)	3 (33)

^a Farms in Chiang Mai and Lampoon provinces were not surveyed.^b Commercial production includes private companies or contract growers.^c Pig owners bought manufactured concentrate feed in the form of pellets for in-house use.

Some antimicrobials remained effective against some resistant bacteria isolated in this study. Ninety percent of the *E. coli* isolates were susceptible to the second or third generation cephalosporins and 70% were susceptible to aminoglycosides (Fig 3). The *Salmonella* spp. isolates were always susceptible to the second or third generation cephalosporins,

aminoglycosides, and quinolones. The *E. faecalis* isolates were always susceptible to vancomycin (glycopeptide class) and tigecycline (glycylcycline class). The *E. faecalis* isolates were often susceptible to amphenicols, beta-lactams, oxazolidinedione, quinolones, and streptogramins.

Cephalosporin resistance in *E. coli*: Thirteen *E. coli* isolates in this study were resistant to the second and third generation cephalosporins (cefoxitin, ceftiofur, and/or ceftriaxone). A subset of these isolates were further characterized and found to contain genes for extended spectrum beta-lactamase (ESBL), including AmpC beta-lactamase (data not shown), which confers resistance to cephalosporin.

Multidrug resistance: Multidrug resistance was detected in 95%, 86%, and 78% of the *Salmonella* spp., *E. faecalis*, and *E. coli* isolates, respectively. *E. coli* and *Salmonella* spp. were most often resistant to four classes of antimicrobials, and "AMP-C-S-SXT-TET" was the most common resistance pattern among 65% of the *Salmonella* spp. isolates and 15% of the *E. coli* isolates (Table 3). *E. faecalis* was most often resistant to five classes of antimicrobials (Fig 2). The most common *E. faecalis* resistance pattern "DA-E-K-RL-S-TET" and permutations with or without streptomycin, kanamycin, or penicillin was found in 41% of the isolates (Table 3).

Table 2 Prevalence of antimicrobial-resistant bacteria in swine feces from farms in three provinces in Thailand

Province	Farms	Number of farms positive for antimicrobial-resistant bacteria (percent positive)		
		<i>Salmonella</i> spp.	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>
Total	51	9 (18)	18 (35)	49 (96)
Chiang Mai	22	8 (36)	4 (18)	22 (100)
Lampoon	8	1 (13)	4 (50)	8 (100)
Nan	21	0	10 (48)	19 (90)

Additional factors from the survey were compared for their effect on the number of antimicrobial resistance traits in *E. coli* from farms. *E. coli* was used as a proxy because it was nearly ubiquitous throughout the study. Factors that were not significant were veterinary oversight ($p = 0.68$) and antimicrobial use frequency ($p = 0.63$). The use of feed purchased from a store was associated with greater numbers of antimicrobial resistance traits ($p=0.01$) than feed not bought from a store. Similarly, a separate question asked if the farms used in-house feed mixed from raw materials, and those that did had significantly lower numbers of resistance traits ($p=0.001$) than farms that did not use in-house feeds.

Discussion

The use of antimicrobials in food animal production creates reservoirs of antimicrobial resistance on farms (Silbergeld et al., 2008). These resistance genes can be readily shared between commensal and pathogenic bacteria (Wright, 2007). Reservoirs of multidrug resistance are present in swine farms of all sizes in Northern Thailand and may be affected by the size of the herd and antimicrobial usage on the farm. Access to antimicrobials as a medicated feed appeared to be an important factor to consider regarding the development of drug resistance in swine farms: operations that purchase store-bought feed (or did not prepare in-house feed from raw ingredients – implying they purchased feed) had significantly more antimicrobial resistance on their farm. We assume, but cannot be certain, that much of the store-bought feed is

Factors related to antimicrobial resistance: Larger farms were related to higher levels of antimicrobial resistance, which was observed in several ways. Firstly, more drug-resistance traits were detected at Lampoon and Chiang Mai provinces than Nan province ($p = 0.032$; pairwise comparisons $p < 0.05$; data not shown). Lampoon and Chiang Mai provinces had larger average herd sizes than Nan province. Secondly, the *E. coli* from surveyed commercial farms had significantly more drug-resistance traits than the *E. coli* from surveyed backyard farms ($p = 0.037$). Lastly, the number of swine on a farm was moderately correlated ($R^2 = 0.44$) with the total number of drug-resistance traits. Strong correlations were observed between farm size and drug-resistant *Salmonella* spp. ($R^2 = 0.84$), and weak correlations were observed between farm size and drug-resistant *E. coli* ($R^2 = 0.26$) and between farm size and drug-resistant *E. faecalis* ($R^2 = 0.14$).

in fact medicated feed. To confirm that antimicrobials were present in feed, we collected commercial feed from six of the larger farms in Nan province, and pooled the feed into a single sample, which was analyzed by liquid chromatography tandem mass spectrometry using EPA Method 1694. We found the following antimicrobials in the pooled sample: ciprofloxacin, erythromycin, lincomycin, penicillin, sulfadimethoxine, sulfamerazine, tylosin, and several tetracyclines (data not shown). We do not know what types of antimicrobials were present in swine feed from other farms, outside of the self-reported antimicrobial usage reported in the survey. Additional feed samples analyses were not performed because of difficulty shipping pig feed from Thailand to the United States for analysis. Commercial medicated feeds present issues relevant to the development of antimicrobial resistance because the farmer has little choice in what drugs will be pre-mixed into the feed and drug dose is dependent upon the amount of feed consumed by the animal (Love et al., 2011).

Herd size was also related to antimicrobial resistance in this study; the farms with larger number of swine had larger reservoirs of multidrug-resistant bacteria, which agrees with findings of herd size and *Salmonella* in Chiang Mai province (Dorn-in et al., 2009). In our survey, the commercial farms used store-bought feed, which was likely medicated, while the backyard farms were more likely to use in-house feeds. At the backyard farms, typically farmers or local veterinarians would inject antimicrobials only if clinical signs of disease were observed in animals, and

the surveyed farmers reported being more likely to administer antimicrobial intermittently or episodically to pigs under the supervision of a local veterinarian, as compared to the commercial farms. While these backyard farms also contained multidrug-resistant bacteria, the rates of resistance were lower than on the commercial farms. It is recommended that farmers administer injectable antimicrobials for a full course (typically 7 d), although in a professional observation by one of the authors (O. Arjkumpa, unpublished observation) sometimes antimicrobials were administered until the animal no longer showed clinical symptoms (around 3 d). This practice should be discouraged.

Regarding *Salmonella* spp., compared to our study, previous studies found a similar or slightly lower prevalence of antimicrobial-resistant *Salmonella* spp. in swine in Thailand, ranging from 1% to about 20% (Hanson et al., 2002; Padungtod and Kaneene, 2006; Pathanasophon et al., 2007; Pulsrikarn et al., 2012). As with other studies in Thailand, our study found that resistance to ampicillin, chloramphenicol, streptomycin, and tetracycline was common among *Salmonella* spp. (Hanson et al., 2002; Padungtod and Kaneene, 2006; Pulsrikarn et al., 2012); in addition, we detected resistance to trimethoprim/sulfamethoxazole. Tetracycline was not reportedly used by the surveyed farmers, however, our independent chemical analyses detected tetracycline in swine feed. Most isolates in our study were resistant to four drugs, which appears to be an increase in resistance traits over a previous work in 2000 to 2003 (Padungtod and Kaneene, 2006). Relevant to the medical community, the *Salmonella* isolates we detected were always susceptible to the second or third generation cephalosporins, aminoglycosides, and quinolones. Cephalosporin susceptibility could be called into question in the future if cephalosporin-resistant commensal *E. coli* (in 10% of the isolates in this study) shared resistance traits with *Salmonella*.

For *E. coli*, we found a higher prevalence of antimicrobial-resistant *E. coli* in swine than previous reports from Northern Thailand (Hanson et al., 2002). The antimicrobial resistance traits for *E. coli* in our study were similar to those previously reported in Northern and Southern Thailand (Hanson et al., 2002; Phongpaichit et al., 2007). ESBL genes were identified among cephalosporin-resistant isolates in this study and although we do not know with certainty if cephalosporins were administered to the swine in this study, others have found the use of cephalosporins in swine production for ESBL producing *E. coli* strains (Cavaco et al., 2008). Future work with swine and ESBL producing bacteria may find a similar situation as poultry operations, where ESBL/Amp-C producing *E. coli* has been detected in workers and constitutes a human health risk for this population (Dierikx et al., 2013).

The *E. faecalis* prevalence was 35% in this study, however, further research is needed to understand why the *E. faecalis* resistance rates were lower in Chiang Mai province than Lampoon or Nan provinces. *E. faecalis* prevalence has not been reported previously for swine in Thailand and the lack of data on *E. faecalis* is perhaps because it is part of normal gut

microbiota, and not a human pathogen outside of hospital-acquired infections. We included *E. faecalis* in our study because it is a gram-positive bacterium and provides additional information about the collection of antimicrobial resistance genes, or resistome, present in swine farms. In this study, more than half of the *E. faecalis* isolates were resistant to five or more drugs, and typically those drugs were clindamycin, erythromycin, streptomycin, sulfamethoxazol, and tetracycline. All *E. faecalis* isolates from the swine in this study were susceptible to vancomycin. In hospitals, vancomycin-resistant enterococci (VRE) is a major concern and can lead to difficult-to-treat wound, bloodstream, and urinary tract infections (Cattoir and Leclercq, 2013).

Several limitations existed in this study. The farm surveys were only administered in Nan province, because these data were collected as part of a separate study (Hinjoy et al., 2013) that did not include Chiang Mai or Lampoon provinces. With these limited survey data, we were unable to identify a relationship between farmer-reported antimicrobial use practices and antimicrobial resistance. The sampling plan from Nan Province was not achieved and samples were also collected from Chiang Mai and Lampoon provinces. The findings from Chiang Mai and Lampoon provinces may not be generalizable outside of those study populations due to the convenience sampling technique employed. Due to limited financial resources, the samples were pooled by farm and no *Salmonella*, *E. faecalis*, and only a limited number of *E. coli* isolates were serotyped, and none of the bacterial isolates were genotyped for confirmation. These limitations affect our ability to make comparisons to existing *Salmonella* strain types known to cause foodborne outbreaks in Thailand. The study only asked questions about the prevalence of antimicrobial-resistant bacteria and we did not have the resources to perform repeated visits to the farms to observe temporal trends.

Controlling antimicrobial-resistant bacteria in Thailand begins on the farm. Antimicrobial-free swine operations have a lower prevalence of multidrug-resistant *Staphylococcus aureus* than operations that use antimicrobials, as measured by the presence of these bacteria in workers noses (Rinsky et al., 2013). In addition to farm-level strategies, food safety extends through the supply chain where contamination persists or increases in slaughterhouses and on retail meats in Thailand (Hanson et al., 2002; Padungtod and Kaneene, 2006; Phongpaichit et al., 2007; Pulsrikarn et al., 2012). These public health challenges require appropriate policies and control measures aimed at reducing antimicrobial use in food animal production to suppress the spread of resistant bacteria originating from farms.

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Table 3 Patterns of antimicrobial-resistance in bacterial isolates in swine feces from three provinces in Thailand

Antimicrobial resistance patterns ^a	N
<i>Salmonella</i> spp.	
AMP-C-SXT-TET	11
AMP-SXT-TET	3
AMP-S-TET	2
<i>Enterococcus faecalis</i>	
C-DA-E-K-LZD-RL-S-TET	1
DA-E-K-QD-RL-S-TET	1
DA-E-K-P-RL-S-TET	3
DA-E-K-RL-S-TET	6
DA-E-P-RL-S-TET	2
CIP-DA-E-S-TET	1
DA-E-QD-RL-TET	1
DA-E-K-S-TET	1
DA-E-RL-S-TET	2
DA-K-RL-S-TET	1
DA-P-RL-S-TET	1
DA-E-RL-S	1
DA-K-S-TET	1
DA-K-S	1
DA-RL-S	1
E-RL-TET	1
DA-E	2
S	1
TET	1
<i>Escherichia coli</i>	
AMC-AMP-C-NA-SXT-TET	2
AMP-C-CIP-CN-CRO-EFT-NA-S-SXT-TET	1
AMP-C-CN-CRO-EFT-FOX-NA-S-TET	1
AMP-C-CN-CRO-EFT-NA-S-SXT-TET	1
AMC-AMP-C-CN-CRO-EFT-S-TET	1
AMC-AMP-CRO-EFT-FOX-S-SXT-TET	1
AMP-C-CN-CRO-EFT-NA-S-TET	1
AMP-C-CN-CRO-EFT-S-SXT-TET	1
AMP-C-CIP-CN-K-NA-S-SXT	1
AMP-C-CIP-CN-K-S-SXT-TET	1
AMP-C-CN-CRO-EFT-SXT-TET	1
AMP-C-CN-EFT-K-S-SXT-TET	1
AMP-C-CIP-NA-S-SXT-TET	3
AMP-C-CN-NA-S-SXT-TET	2
AMP-C-CIP-K-NA-S-TET	2
AMP-C-CIP-NA-S-SXT-TET	5
AMP-C-CIP-K-NA-S	1
AMP-C-CRO-EFT-S-TET	1
AMP-C-EFT-S-SXT-TET	1
AMP-C-K-NA-S-SXT-TET	3
AMP-C-K-NA-S-TET	1
AMP-C-K-S-SXT-TET	2
AMP-C-NA-S-SXT-TET	1
AMP-C-CIP-NA-S-TET	1
AMP-C-CN-K-NA-TET	1
AMP-C-CN-K-SXT-TET	1
AMP-C-CIP-CN-K-NA-TET	1

Table 3 (Cont.)

Antimicrobial resistance patterns ^a	N
AMP-D-K-NA-SXT-TET	2
AMP-EFT-NA-SXT-TET	1
C-CIP-K-NA-S-TET	1
AMP-C-CN-SXT-TET	2
AMP-C-K-SXT-TET	4
AMP-C-NA-SXT-TET	2
AMP-CIP-NA-S-TET	4
AMP-CIP-NA-SXT-TET	1
AMP-FOX-S-SXT-TET	1
AMP-K-S-SXT-TET	2
AMP-C-S-SXT-TET	8
AMP-CN-S-SXT	1
AMP-K-NA-TET	1
AMP-C-NA-TET	1
AMP-C-S-SXT	1
AMP-C-S-TET	1
AMP-C-SXT-TET	19
AMP-S-SXT-TET	7
AMP-C-TET	1
AMP-NA-TET	1
AMP-S-TET	6
AMP-SXT-TET	1
S-SXT-TET	1
AMP-C	1
AMP-TET	7
AMP	1
S	1
TET	6

^a Acronyms for antimicrobials are as follows: amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (C), ciprofloxacin (CIP), gentamicin (CN), ceftriaxone (CRO), clindamycin (DA), erythromycin (E), ceftiofur (EFT), cefoxitin (FOX), kanamycin (K), linezolid (LZD), nalidixic acid (NA), penicillin (P), quinupristin/dalfopristin (QD), sulfamethoxazole (RL), streptomycin (S), trimethoprim/sulfamethoxazole (SXT) tetracycline (TET), tigecycline (TGC), and vancomycin (V).

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บทคัดย่อ

การใช้ยาและการดื้อยาปฏิชีวนะของเชื้อ *Salmonella spp.*, *Escherichia coli* และ *Enterococcus faecalis* ในสุกรในพื้นที่ภาคเหนือของประเทศไทย

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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อเพาะแยกเชื้อแบคทีเรียที่ดื้อยาปฏิชีวนะในฟาร์มสุกรที่เลี้ยงแบบอุตสาหกรรมและแบบเลี้ยงหลังบ้านในพื้นที่ภาคเหนือของประเทศไทยและจำแนกการใช้ยาปฏิชีวนะในฟาร์มสุกร โดยทำการศึกษาฟาร์มสุกรในพื้นที่จังหวัดเชียงใหม่ จังหวัดลำพูนและจังหวัดน่านจำนวน 51 ฟาร์มในระหว่างเดือนกุมภาพันธ์ถึงเดือนตุลาคม 2554 โดยเก็บตัวอย่างอุจจาระเพื่อนำไปเพาะแยกเชื้อแบคทีเรีย (*Salmonella spp.*, *Escherichia coli*, *Enterococcus faecalis*) และทำการทดสอบการดื้อยาของเชื้อแบคทีเรียร่วมด้วยรวมทั้งทำการสำรวจเกี่ยวกับระบบการผลิต การจัดการฟาร์ม สุขภาพสัตว์ และการใช้ยาปฏิชีวนะภายในฟาร์มในพื้นที่จังหวัดน่าน (20 ฟาร์ม) พบว่าระดับความชุกรายฟาร์มของการดื้อยาของเชื้อ *E. coli*, *E. faecalis* และ *Salmonella spp.* มีค่าเท่ากับ 96, 35 และ 18% โดยฟาร์มสุกรที่เลี้ยงแบบอุตสาหกรรมจำนวน 4 ฟาร์ม (จำนวนสุกรเฉลี่ย 58 ตัว/ฟาร์ม) มีการใช้ยาปฏิชีวนะเป็นประจำในสุกรเท่ากับ 75% (>50% ของช่วงชีวิตสุกร) ส่วนในฟาร์มสุกรแบบเลี้ยงหลังบ้านจำนวน 16 ฟาร์ม (จำนวนสุกรเฉลี่ย 6 ตัว/ฟาร์ม) มีการใช้ยาปฏิชีวนะเป็นครั้งคราวในสุกร ประมาณ 89% (10-50% ของช่วงชีวิตสุกร) หรือ มีการใช้ยาปฏิชีวนะเป็นบางช่วง (<10% ของช่วงชีวิตสุกร) และพบว่ามีฟาร์มจำนวน 1 ฟาร์มที่ใช้ยาปฏิชีวนะเป็นประจำในสุกร นอกจากนี้ยังพบว่าขนาดฟาร์มมีความสัมพันธ์เชิงบวกกับระดับปานกลางกับจำนวนของการดื้อยาปฏิชีวนะในฟาร์ม ($R^2 = 0.44$) การศึกษาครั้งนี้แสดงให้เห็นว่าพัฒนาการดื้อยาปฏิชีวนะหลายชนิดอย่างกว้างขวางในฟาร์มสุกรทุกขนาดในภาคเหนือของประเทศไทยและอาจมีผลเกี่ยวกับขนาดฟาร์มและวิธีการใช้ยาปฏิชีวนะภายในฟาร์ม

คำสำคัญ: ยาต้านจุลชีพ *E. coli* แบคทีเรียที่ดื้อยาปฏิชีวนะหลายชนิด *Salmonella* สุกร

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