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Antimikrobiell resistens hos *Escherichia coli* i små grisbesättningar i nordöstra Thailand

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SUMMARY

Antimicrobial resistance (AMR) is a fast growing problem throughout the world, and as a consequence effective treatment of various infections is jeopardized. This results in prolonged illness and increased mortality amongst patients as well as increased health care costs. It is known that excessive usage of antimicrobial drugs contributes to the development of antimicrobial resistance. There have also been reports of resistant bacteria spreading from animals to human. However, the full magnitude of the problem worldwide is still not known. In a joint effort to combat AMR, the Food and Agricultural Organization of the United Nations (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE) therefore encourages countries to develop harmonized AMR-monitoring programs to map the AMR in food animals.

The pig industry in Southeast Asia has been steadily growing the last decades; however the knowledge about the occurrence of AMR is poor. In Thailand there have been studies that map AMR in pigs; however there is a lack of standardization and harmonization, which makes national data difficult to interpret.

The purpose of this study was to investigate the occurrence of antimicrobial resistance in small-scale swine herds in Khon Kaen Province in the northeast of Thailand, using intestinal *Escherichia coli* as indicator bacteria. Furthermore the purpose was also to compare the result from this study with data from similar studies from Thailand and other countries.

Twenty-five farms were visited, which housed a maximum of twenty sows each. To help gain insight in pig farming in Thailand and help identify possible factors affecting AMR, the person responsible for the pigs at each farm was asked to fill in a questionnaire with questions about the farm, husbandry, antibiotic usage, vaccination regimes etc.

On each farm three samples were collected in the form of rectal swabs from three different healthy sows. A total of 69 samples were collected. From each sample *E. coli* was cultured and tested for antimicrobial susceptibility using a standardized broth microdilution method to determine minimum inhibitory concentrations (MICs).

Resistant isolates of *E. coli* was found on all farms. Resistance against tetracycline (75.3% of the isolates), ampicillin (69.6%) and sulfamethoxazole (60.8%) were the most common. Multiresistance could be seen in 69.5% of the isolates. One suspected extended spectrum beta-lactamase producing (ESBL)-isolate was found. Statistical analyses to identify risk factors for AMR were difficult to perform due to the small number of observations and those made must therefore be considered to lack in statistical power.

When compared to OECD countries the level of AMR was generally higher in this study. When compared to studies from Thailand made on larger farms, the AMR levels were generally lower in this study.

Antimicrobial resistance seems to be common in small-scale swineherds in north-eastern Thailand. However, a national standardized and harmonized monitoring program is needed to fully evaluate the situation.

SAMMANFATTNING

Antimikrobiell resistens (AMR) är ett problem som växer snabbt runt om i världen. Som en konsekvens av detta ökar risken att olika infektioner inte längre går att behandla effektivt. Detta resulterar i förlängd sjukdomstid och ökat antal dödsfall samt ökade kostnader för sjukvård. Det är känt att överdriven användning av antimikrobiella läkemedel bidrar till utvecklingen av antimikrobiell resistens. Det finns även rapporter om resistenta bakterier som sprider sig från djur till människor. Dock är det ännu inte klarlagt hur stort problemet är globalt. Med målsättning att motverka AMR, har Förenta nationernas livsmedels- och jordbruksorganisation (FAO), Världshälsoorganisationen (WHO) och Världsorganisationen för djurhälsa (OIE) gått samman i frågan och uppmuntrar därför länder att utveckla och införa standardiserade AMR-övervakningsprogram för livsmedelsproducerande djur.

Grisindustrin i sydöstra Asien har växt under de senaste decennierna. Dessvärre är kunskapen om utbredningen av AMR dålig. I Thailand har studier som kartlägger AMR gjorts på grisar, men då standardiserade metoder inte använts är den nationella datan svårtolkad.

Syftet med denna studie var att undersöka förekomsten av AMR i små grisbesättningar i Khon Kaen-provinsen i nordöstra Thailand, genom att använda *Escherichia coli* i tarmen som indikatorbakterie. Vidare var syftet att jämföra resultaten från denna studie med data från liknande studier från Thailand och andra länder.

Tjugofem gårdar med max tjugo suggor vardera besöktes. För att få ökad insikt i grisproduktion i Thailand och för att kunna finna möjliga faktorer som påverkar AMR fick ansvarig person på gården fylla i ett frågeformulär. Detta innehöll frågor rörande gården, skötseln, antibiotikaanvändning, vaccinationsrutiner etc.

På varje gård togs tre prov i form av rektalsvabbar från tre olika friska suggor. Totalt samlades 69 prover in. Från varje prov isolerades *E. coli* och därefter undersöktes isolatens känslighet för olika antibiotika. Detta gjordes genom att använda en standardiserad buljong-mikrodilutionsmetod för att bestämma MIC (minimum inhibitory concentration).

Resistenta isolat av *E. coli* sågs på alla gårdar. Resistens mot tetracyklin (75,3 % av isolaten), ampicillin (69,6 %) och sulfamethoxazole (60,8 %) var vanligast. Multiresistens sågs hos 69,5 % av isolaten. Ett misstänkt ESBL(extended spectrum beta-lactamase)-producerande isolat hittades. Statistiska analyser för att indentifiera riskfaktorer för AMR var svåra att genomföra då antalet observationer i de flesta fall var för få. De analyser som gjorts måste därmed anses sakna statistisk ”power”.

Jämfört med OECD-länder var förekomsten av AMR generellt sett högre i denna studie. Vid jämförelse med andra studier från Thailand utförda på större gårdar, var förekomsten av AMR generellt sett lägre i denna studie.

Sammantaget tycks antimikrobiell resistens vara vanligt förekommande i små grisbesättningar i nordöstra Thailand. Det behövs emellertid ett nationellt standardiserat övervakningsprogram för att fullt ut kunna utvärdera situationen.

CONTENT

ABBREVATIONS

AMR – Antimicrobial resistance

ECOFF - Epidemiological cut-off value

EFSA - European Food Safety Authority

EUCAST – European Committee on Antimicrobial Susceptibility testing

FAO – Food and Agriculture Organization of the United Nations

MIC – Minimum Inhibitory Concentration

OIE – World Organization for Animal Health

PCU – Population Correction Unit

WHO – World Health Organization

Abbreviations for antibiotic substances

Gm – Gentamicin

Km – Kanamycin

Sm – Streptomycin

Ak – Amikacin

Am – Ampicillin

Ctr – Ceftriaxone

Cox – Cefoxitin

Cef – Cefuroxine

Cti – Ceftiofur

Ctx – Cefotaxime

Caz – Ceftazidine

TrSu – Trimethoprim-sulfamethoxazole

Tm – Trimethoprim

Su – Sulfamethoxazole

Sua – Sulfonamides

Cm – Chloramphenicol

Ff – Florfenicol

Cs – Colistin

Ci – Ciprofloxacin

Nal – Nalidixic Acid

Tc – Tetracycline

INTRODUCTION AND LITTERATURE REVIEW

Antimicrobial resistance (AMR) throughout the world is a problem which has been growing for several decades. It is, according to the World Health Organization (WHO), a problem so severe that it threatens the achievements of modern medicine. As the problem grows, the effective treatment of various infections is jeopardized. Although the magnitude of the problem worldwide is still largely unknown, it is clear that AMR might lead to prolonged illness and increased mortality in patients, as well as increased health care costs (WHO, 2014).

In a report in 2013, WHO estimated that hospital-acquired infections with multiresistant bacteria annually causes the death of around 30 000 people in Thailand, 25 000 people in the European Union, 23 000 people in the United States of America and 80 000 people in China. In addition, it is estimated that the cost due to these antibiotic-resistant infection is 2000 million USD in the US and 1500 million EUR in the EU (WHO, 2013).

Chantziaras *et al.,* (2013) showed in a study that the use of veterinary antibiotics in agriculture has been linked to the development of resistance in animals. Evidence suggests that transfer of resistance from animals to humans is possible and thus might cause harm to the public health (Marshall & Levy, 2011).

Thailand has a large swine industry, which has improved rapidly during the last decades. There is no reliable information about the sales and usage of antibiotics in the country, (UN Food and Agriculture Organization (FAO), 2014a). However Tantasuparuk & Kunavongkrit (2015) report an increased misuse of antibiotics, vaccines and off-label chemicals. Likewise, there is lacking information about the current status on AMR in the country and no harmonized AMR-monitoring program on a national level, although it is currently under development (FAO, 2014b).

The purpose of this study was to investigate the occurrence of antimicrobial resistance in small-scale swine herds in Khon Kaen Province in the northeast of Thailand, using *Escherichia coli* as indicator bacteria. At the time this study was carried out, another study was performed in the same area with the same purpose but with focus on medium-scale farms instead (Halje, forthcoming). The purpose was also to compare the findings with data from similar studies from Thailand and other countries. According to the World Health Organization (2014), only a couple of EU countries, USA and Canada have harmonized programs which continuously evaluate the levels of AMR in food producing animals. Therefore studies from some of these OECD countries were chosen for comparing the data.

Pig production

Throughout the world, the demand for meat is growing. This has led to an increase in the number of animals raised and slaughtered each year. Fast growing breeds of pigs with an efficient feed conversion rate are thus likely to account for a great share of the livestock market in the future. In recent decades the pig production throughout the world has greatly been commercialized, with fewer, larger farms raising a greater number of animals.

Particularly in the developed world, these large-scale pig operations have more or less outrivaled the traditional way of raising pigs, with a few exceptions such as organic pig farms and other niche markets (FAO, 2014c).

In the developing world however, half of the pig population is still kept in small-scale farming systems. Raising pigs in these systems can be done with little initial investment and doesn't necessarily require any agricultural land. In addition to providing the families with meat and sales income, the pigs also acts as a "bank" where the wealth can be accessed whenever money is needed, such as for new investments or health care fees. Furthermore it provides an income for women, which helps them strengthen their position in the community, provides job opportunities for family members and is overall considered a low risk investment with quick returns (FAO, 2014b; FAO, 2011).

Thailand, along with some of its neighboring countries, has a large swine industry. Since Thailand joined the World Trade Organization in 1995, the pig and pork sectors in the country have improved fast. In 2013 16.2 million fatteners were produced and the standing population of pigs of all ages was 9.51 million. During the last five decades Thailand has seen a shift in pig production where the number of small and medium-scale farms has declined in favor of larger farms with more animals. Nowadays small and medium-scale farms possess 40% of the total pig population, as opposed to 70% before 1970. Small-scale farming is still quite common with 94.15% of the total number of households (210,978) raising less than 50 pigs each in 2013 (Tantasuparuk & Kunavongkrit, 2015).

Antimicrobial resistance

Development of AMR

Antimicrobial resistance occurs through mutations in the bacteria's chromosome or via gene transfer mechanisms where genetic material is exchanged between bacteria (Furuya & Lowy, 2006). It is however not a new phenomenon. Most of the antimicrobial drugs that we use today are substances that are naturally produced by microorganisms or are modifications of these substances. Research shows that some bacteria developed strategies to evade these substances millions of years ago. Resistance against synthetic antimicrobials (sulphonamides and quinolones) has also emerged since they were introduced and is today present throughout the world (Holmes *et al.*, 2015).

Bell *et al.* (2014) showed that there is a positive link between human antibiotic consumption and the development of antibiotic resistance in humans. Likewise, Chantziaras *et al.*, (2013) showed that there are remarkably strong indications of a positive link between veterinary antibiotic usage and the development of resistant bacteria in animals, which was also stated by the FAO on World Veterinary Day 2012 (FAO, 2015). What is still somewhat under debate though is the link between antibiotic use in agriculture and the rise of AMR-bacteria in humans and how strong the connection is (Marshall & Levy, 2011).

Chang *et al.* (2014) describe three different mechanisms for how antibiotic use in agriculture could be potentially harmful for humans. 1: Direct infection with a pathogen from an animal or contaminated food, but without further transmission between humans. 2: A resistant microbe infects or colonizes a human like mentioned above, which is then followed by transmission from human to human. In some cases humans may become ill. 3: Resistance genes arise in agricultural settings, which are then transferred to human pathogens.

While there are several documented cases of mechanism one and two, it has been more difficult to study the effects of mechanism three. This is due to the fact that genes may mutate over time while passing through different hosts until they no longer resemble the original gene, which makes tracking difficult (Chang *et al.*, 2014; Marshall & Levy, 2011). However, despite these difficulties there are documented cases where gene transfer between bacteria originating from different species has occurred (Wang *et al.*, 2012; Kruse & Sørum, 1994).

Mechanisms of AMR

Different protective mechanisms have evolved that helps the bacteria evade antimicrobial drugs, such as preventing the drug from entering the bacteria, increased transportation of the drug out of the bacteria (efflux pumps), alteration of the drugs target molecule and producing enzymes that modifies or destroys the drug (Holmes *et al.*, 2015).

Efflux mediated resistance

Being an important AMR mechanism, drug efflux pumps make it possible for the bacteria to evade a drug by transporting it out of the bacteria. It is a key mechanism of AMR, especially in Gram-negative bacteria. Drug efflux pumps can be found in bacteria from human, animal, plant and environmental origin and the number of pumps described has increased over the years. This is a major concern, since multidrug efflux pumps are common and a single pump may result in resistance to a number of drugs (Li & Nikaido, 2009).

Modified target sites

Different types of antimicrobials have different target sites i.e. penicillin-binding-proteins (PBP) in the cell wall (beta-lactam antibiotics), DNA-gyrase/Topoisomerase IV (fluoroquinolones), 16S rRNA (aminoglycosides) etc. As these target sites are often involved in vital cellular functions, the bacteria cannot simply get rid of them to avoid the antimicrobials. Instead, often bacteria modify the target site so that the function remains intact, whilst reducing the susceptibility to the antimicrobial (Lambert, 2005). A current example of this is Methicillin-resistant *Staphylococcus aureus* (MRSA). The gene *mecA* is acquired and integrated into the chromosome. When expressed, this gene encodes for a penicillin-binding protein 2a (PBP2a), which substitutes other PBPs. This enables the bacteria to survive the antibiotic, due to the fact that PBP2a has a lower affinity to beta-lactams than regular PBPs (Lowy, 2003).

ESBLs – Extended Spectrum Beta-Lactamases

Extended spectrum beta-lactamases are a group of enzymes produced by different kinds of bacteria. The classical definition of ESBLs was set in 1995, as beta-lactamases with the ability to hydrolyze and thus inactivate beta-lactam antibiotics of the penicillin group, cephalosporins of the third generation as well as monobactams (aztreonam), but which are inhibited by clavulanic acid (Lee *et al., 2012*). This is a definition that is still widely used today, but has proved to be somewhat limiting as the knowledge about this group of enzymes has improved. There is no clear consensus in the definition of ESBLs and different ways of categorizing have been proposed (Lee *et al., 2012*). Giske *et al*. (2009) proposed a way to categorize the ESBLs into three different groups: Classical ESBLs (as mentioned above), miscellaneous ESBLs with a range of different additional traits and lastly $\mathrm{ESBL}_{\mathrm{CARBA}}$ with the ability to hydrolyze carbapenems as well as other beta-lactam antibiotics.

Bacteria that produce $\mathrm{ESBL}_{\mathrm{CARBA}}$ have spread quickly throughout the world and have been found in both humans and animals (Fischer *et al.,* 2012; WHO, 2014). This is troublesome, since there are very few other antibiotics these bacteria are susceptible to and thus treatment options are slim (WHO, 2014).

AMR throughout the world

Antimicrobial resistance surveillance

Because of differences in methodology and laboratory procedures throughout the world, comparison of data on AMR in food-producing animals between countries is difficult and sometimes impossible. According to the WHO there are only a limited number of countries in the world today with harmonized AMR surveillance programs in food-producing animals, despite the fact that there have been several recommendations over the last decades. Only in some EU countries, USA and in Canada there are continuous programs that allow for comparison of data. FAO, WHO and the World Organization for Animal Health (OIE) have joined together in an effort to combat AMR and have pointed out the need for a more harmonized global standard for AMR surveillance in the food chain (WHO, 2014).

Thailand

Antimicrobial resistance in livestock is routinely monitored in Thailand, but there is lack of harmonization which makes national data difficult to interpret. The National Institute of Health, a part of the Department of Livestock Development (DLD) is the institute that oversees the AMR-monitoring on a national level. A project to harmonize the AMR-monitoring across the country is currently in development by the DLD, partly inspired by the AMR-monitoring programs from the USA and The European Union. Some problems that have to be addressed include lack of harmonization and standardization in AMR-monitoring, unregulated usage of antimicrobials, lack of knowledge regarding antibiotic usage on farms and untrained personnel (FAO, 2014b).

According to a review article released by the FAO (2014b), the antimicrobial resistance in *E. coli* from livestock is well studied in East, South and Southeast Asia and resistance is widespread. However, only two studies on *E. coli* AMR from pigs in Thailand were included in this review article and no information about these studies was provided, which hinders further evaluation.

A study in 2008 investigated AMR in *E. coli* from pigs in the north-eastern parts of Thailand (Jiwakanon *et al.*, 2008). Fecal samples were collected between 2003 and 2005 and *E. coli* was isolated from 338 samples. The isolates were tested for susceptibility to 15 types of antimicrobial drugs (table 17). Due to translation shortcomings, it is not possible to assess the methods of isolation or AMR-testing nor how the samples were collected.

Another Thai study in 2014 investigated the occurrence of AMR-bacteria with a focus on ESBL-producing *E. coli* in healthy food animals in a northern and eastern province in Thailand (Boonyasiri *et al., 2014*). A total of 400 samples in the form of rectal swabs were collected from randomly chosen healthy pigs (age not specified). There was no information regarding the sizes of the farms. A disk diffusion method was used to determine the antibiotic susceptibility (table 17).

Europe

In 2013 the European Commission decided that from 2014 it would become mandatory for member states of the European Union to monitor AMR in *E. coli*, *Salmonella* and *Campylobacter jejuni* in different food producing animal populations with regular intervals. It was also decided that microdilution methods are the standardized way for testing antimicrobial susceptibility, followed by the use of Epidemiological cut-off (ECOFF) values to interpret the results. These values are provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (European Commission 2013/652/EU of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria).

A report from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) included 1954 isolates from 10 different member states of the European Union. The isolates originated from either fattening pigs or breeding animals (seven and one country, respectively) or unspecified (two countries). The majority of the isolates were collected as part of the member states' national AMR monitoring program from healthy slaughter pigs at the slaughterhouse. Three countries did not account for sampling stage, sample type or sampling context. ECOFFs were used to interpret the antimicrobial susceptibility data in accordance with Decision 2013/652/EU (EFSA & ECDC, 2015). The results can be seen in table 17.

Sweden

The Public Health Agency of Sweden and the National Veterinary Institute each year publish a report that includes data regarding consumption of antibiotics and occurrence of antibiotic resistance in humans and animals in Sweden (Swedres-Svarm, 2015). This is a collaboration between relevant sectors in Sweden and includes zoonotic pathogens, human clinical isolates, animal clinical isolates and, in accordance with the European Commission's Decision 2013/652/EU of 12 November 2013, indicator bacteria *E. coli* and *Enterococcus* from healthy animals.

The last year Sweden included indicator *E. coli* from pigs in the report was 2011. Samples in the form of colon content were collected from healthy pigs at slaughter. Each sample represented a unique herd. The methods used to test the antimicrobial susceptibility of the isolates were the same as in this thesis (see Material and methods) (SVARM, 2012). The results can be seen in table 16 and 17.

Denmark

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) was established in 1995. The National Food Institute, the National Veterinary Institute and Statens Serum Institut are working together to monitor the consumption of antimicrobial agents in food animals and humans and the occurrence of antimicrobial resistance in bacteria from food animals, meat and humans. Like Sweden the antimicrobial resistance monitoring is based on zoonotic bacteria, pathogenic bacteria in humans and animals as well as indicator bacteria *E. coli* and *Enterococcus* (DANMAP, 2015).

In the report of 2014, a total of 209 samples in the form of caecal content were collected at slaughter plants throughout Denmark. It is unclear whether every sample represented a unique herd. A MIC microbroth dilution method in accordance with the European Committee for Standardization (2007) was used to test the antimicrobial susceptibility. The results can be seen in table 17 (DANMAP, 2015).

Canada

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is a national program established in 2002. One of its objectives is to monitor trends in antimicrobial resistance and antimicrobial use in humans and animals.

Farms that produced a minimum of 2000 market pigs per year were selected and visited once per year. Pooled fecal samples were collected from six pens from pigs close to slaughter (>80kg). A total of 1573 samples were collected. The farms were spread out evenly throughout the country in proportion to the total number of produced fattening pigs. The methods used to test the antimicrobial susceptibility of the isolates were much like the one used in this report (see Material and methods). Results from the report of 2013 are shown in table 17 (CIPARS, 2015a).

Antibiotic consumption

The consumption of antibiotic drugs worldwide is increasing. Van Boeckel, *et al.,* (2015) were the first to do a quantitative assessment of the global antibiotic consumption in livestock.

In 2010 it was estimated to 63.151 ± 1560 tonnes and is projected to increase with 67% to 105.596 ± 3605 tonnes by the year 2030. The rising incomes in low- and middle-income countries has driven up the demand for meat and therefore intensive production systems that are able to produce meat more efficiently has increased. These systems require antibiotics for the animals to stay healthy, and therefore rising incomes in such countries are driving an increase in antibiotic consumption in livestock. Indeed, in Brazil, Russia, India, China and South Africa (BRICS-countries) the antibiotic consumption in livestock is estimated to increase up to 99% in 2030.

Thailand

The Department of Livestock Development (DLD) in cooperation with the Food and Drug Administration (FDA), Ministry of Public Health regulates the use of veterinary drugs. The FDA is responsible for licensing and registration of veterinary drugs and the DLD is responsible for surveillance and control of the usage of veterinary drugs and also to list drugs and chemicals that are not allowed in food producing animals. Currently all antibiotic use for growth promotion in food animals is banned in the country (FAO, 2014b).

FAO (2014a) reports that usage of antimicrobial drugs based on the personal experiences of the farmers without any diagnostic tests are common. This indicates that the usage is poorly supervised and that it’s easy for the farmers to obtain antimicrobial drugs. However, no large study has been done regarding the antibiotic usage and sales in south-eastern Asia, and so there are still uncertainties about the true volumes used.

Europe

Growth-promoting antibiotics in the feed are forbidden in the European Union since 2006, due to the risk of development of cross-resistance to drugs used in human and veterinary medicine (Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition).

A report by European Medicines Agency (EMA) & European Surveillance of Veterinary Antimicrobial (ESVAC) (2015) compared 26 different European countries and their sales of antimicrobial agents in 2013. The total amount of antimicrobial drugs sold for veterinary use in food producing animals in these countries amounted to 8059.2 tonnes of active ingredients.

The differences in animal demographics play a role in the amount of antimicrobial drugs sold in each country. To account for this EMA & ESVAC uses Population Correction Units (PCU), which is measured as 1 PCU = 1kg of animal weight.

An average of 109.7 mg antimicrobial agents/PCU was used in these 26 countries, with huge differences between countries. The countries that used the most and least antimicrobial drugs were Cyprus and Norway with 425.8 and 3.7 mg/PCU respectively.

Sweden

According to the 2014 Svarm-report, 10,271 kg active substance of antimicrobial drugs was reportedly sold for veterinary use in Sweden 2014. Of these 2,883 kg were used in the Swedish pig industry. There are, however, some uncertainties regarding these numbers; as the Swedish pharmacy market was reregulated some years ago and the data on consumption since are assumed to be less complete than before. Three quarters of the antimicrobial drugs sold were injectable products. The most commonly sold drug for use in pigs was benzylpenicillin with 45% of the total sale. The overall consumption of antimicrobial drugs in pigs has been reportedly quite stable during the last five year, however there has been a shift in that the sales for products intended for group medication has decrease, while products for individual treatment has increased (Swedres-Svarm, 2015).

According to EMA & ESVAC (2015) the total consumption of antimicrobial agents in food producing animals in Sweden in 2013 was 12.6 mg/PCU. This is the third lowest usage of antimicrobial drugs in food producing animals among the countries in the report. Only Norway and Iceland had a lower rate (3.7 and 5.3 mg/PCU respectively).

According to EU Regulation No 1831/2003, the use of growth promoting antibiotics in the feed is prohibited in Sweden, but a national ban was introduced already in 1986 (Cogliani *et al*., 2011).

Denmark

Like in Sweden, growth promoting antibiotics in the feed is prohibited in Denmark according to EU Regulation No 1831/2003. This started during the years 1994-1999 when many feed administered antimicrobial agents were discontinued by the country’s own initiative. Likewise a voluntary ban of the use of cephalosporins in pigs and dairy cattle was introduced more recently (DANMAP, 2015).

According to the Danish report of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark (DANMAP, 2015), the total consumption of antimicrobial agents in veterinary medicine in Denmark 2014 was amounted to 108.5 tonnes of active ingredients. The use of antimicrobial agents in pigs amounted to 82.5 tonnes (76% of the total).

According to the EMA & ESVAC (2015) report, Denmark used 44.9 mg antimicrobial agents/PCU in 2013.

Canada

According to the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), a total of 1.6 million kilograms of antibiotics were distributed for sale for use in animals in Canada in 2012.

In contrast to the European Union, antibiotics in the feed for disease prevention and growth promotion are allowed in Canada. Antibiotics in the feed were used by 82% of the farms. The most common reasons for the use of antimicrobial use in the feed were for disease prevention (49%) or growth promotion (40%). Canada used ca. 160 mg antimicrobial agents/PCU for animals in 2012 according to CIPARS (2015b).

MATERIAL AND METHODS

The method used for testing the antimicrobial susceptibility of the isolates in this project is in accordance with the standard method established by the European Committee for Standardization (CEN) in November 2006 (European Committee for Standardization, 2007).

Farms and logistics

A total of 25 farms located in the surroundings of Khon Kaen were included in the study. The inclusion criterion was that each farm had a maximum of 20 sows. With the help of the local supervisor, the farms were selected from a list provided by the local veterinary service officer. The local supervisor also arranged for transportation in university vans and for 5th or 6th year Thai veterinary students to ride along to act as translators.

Questionnaire

At every farm the person responsible for the pigs was asked to fill in a questionnaire with questions about the farm, husbandry, antibiotic usage, vaccination regimes etc. (Appendix 1). This was to help gain insight in pig-farming in Thailand and the possibility to identify possible protective or risk-factors for AMR.

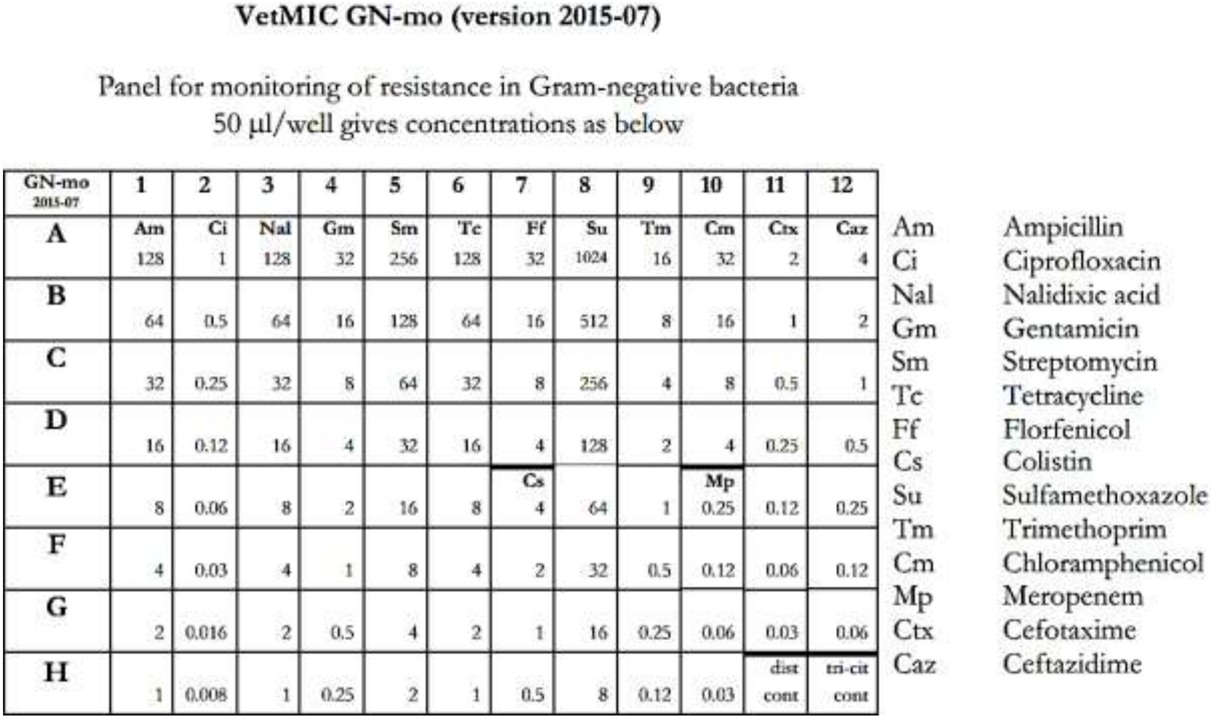
Sample collecting

The samples were collected during the weekends over the course of 4 weeks in September. On each farm, three samples were collected in the form of rectal swabs from three randomly picked healthy sows. The swabs were performed by either the author or a colleague (Halje, forthcoming). If a farm did not have three healthy sows, as many samples were taken as there were healthy sows. A total of 69 samples were collected. The swabs were put in tubes containing Amie's transport medium for the transport back to the laboratory at the Veterinary Faculty, Khon Kaen University. The duration of the transport was between 1 and 6 hours. Tubes were stored for a maximum of 48 hours at 2-4°C before work in the laboratory began.

AMR detection

To isolate *E. coli* bacteria each swab was streaked on a MacConkey agar plate and incubated in 44°C overnight. Then colonies that morphologically looked like *E. coli* were sub-cultured individually on blood agar plates and incubated in 37°C overnight. Finally the resulting bacterial growth was incubated in Motility-Indole-Lysine (MIL) medium in 37°C overnight. Kovac's reagent was added to test the isolate for production of tryptophanase (indole-test). A positive indole test was used to confirm that it was an isolate of *E. coli*. Only one confirmed *E. coli* isolate per animal was included in the antimicrobial susceptibility testing.

To test the antimicrobial susceptibility of the isolates, the minimum inhibitory concentrations (MICs) were determined using a broth microdilution method (European Committee for Standardization, 2007). VetMIC GN-mo panels (Version 2015-07) manufactured by the Swedish National Veterinary Institute were brought along to Thailand. Each VetMIC panel consists of 8 x 12 wells containing different dried antibiotics in serial twofold dilutions. In total, 14 different antibiotics are included (Figure 1).



VetMIC GN-mo (version 2015-07)

Panel for monitoring of resistance in Gram-negative bacteria
50 µl/well gives concentrations as below

GN-mo 2015-07	1	2	3	4	5	6	7	8	9	10	11	12
A	Am 128	Ci 1	Nal 128	Gm 32	Sm 256	Tc 128	Ff 32	Su 1024	Tm 16	Cm 32	Ctx 2	Caz 4
B	64	0.5	64	16	128	64	16	512	8	16	1	2
C	32	0.25	32	8	64	32	8	256	4	8	0.5	1
D	16	0.12	16	4	32	16	4	128	2	4	0.25	0.5
E	8	0.06	8	2	16	8	Cs 4	64	1	Mp 0.25	0.12	0.25
F	4	0.03	4	1	8	4	2	32	0.5	0.12	0.06	0.12
G	2	0.016	2	0.5	4	2	1	16	0.25	0.06	0.03	0.06
H	1	0.008	1	0.25	2	1	0.5	8	0.12	0.03	dist cont	tri-cit cont

Am	Ampicillin
Ci	Ciprofloxacin
Nal	Nalidixic acid
Gm	Gentamicin
Sm	Streptomycin
Tc	Tetracycline
Ff	Florfenicol
Cs	Colistin
Su	Sulfamethoxazole
Tm	Trimethoprim
Cm	Chloramphenicol
Mp	Meropenem
Ctx	Cefotaxime
Caz	Ceftazidime

Figure 1: *The VetMIC GN-mo panel used for antimicrobial susceptibility. In each column the concentration of the antibiotic increases twofold for each well in the direction H to A. The wells containing meropenem later proved to be faulty and wasn't included in the study.*

The broth used was sterile cation adjusted Mueller Hinton broth (CAMHB) with pH 7.2-7.4. A plastic loop was used to collect 3-5 colonies of *E. coli* from a blood agar plate. These were then suspended in 5 ml CAMHB and incubated in 37°C for 1 h 50 min to reach a desired concentration of 10^8 CFU/ml. To confirm the concentration the suspension was visually assessed to have a turbidity of 0.5 McFarland, after which 10 µl of the suspension was transferred to 10 ml CAMHB to obtain a final inoculum density of approximately 5×10^5 CFU/ml. The density was regularly verified by taking 10 µl of the inoculum and diluting it in 10 ml 0.9% saline. Of this dilution 100 µl was then spread evenly on a blood agar plate and incubated in 37°C overnight. The resulting growth was then checked to be 10-100 CFU.

Each well was filled with 50 µl of the inoculum, sealed with plastic film and incubated in 36°C for 17 hours. The wells were then inspected and the lowest concentration inhibiting visible growth was read as the MIC (Swedres-Svarm, 2015).

To secure the quality of the MIC tests, control stain *E. coli* CCUG 17620 (ATCC 25922) was included in every round of tests.

Interpreting the MICs

Epidemiological cut-off values (ECOFFs) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used to interpret the MICs of the antibiotics. An isolate is classified as resistant if the MIC exceeds the ECOFF (http://www.eucast.org).

ECOFFs are used to find isolates with acquired reduced susceptibility, but do not provide information whether or not an isolate will respond to antimicrobial treatment. An isolate that is classified as resistant in this report is therefore not necessarily clinically resistant.

Multiresistance

In this thesis the definition of multiresistance is in accordance with the definition proposed by Magiorakos *et al.* (2012). In their paper, multiresistance is defined as an isolate with acquired resistance against three or more different classes of antibiotics. This is also the way multiresistance is defined in the Swedish Veterinary Antibiotic Resistance Monitoring (Svarm) report (Swedres-Svarm, 2015).

Statistical analyses

Statistical analyses were conducted in SAS software 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics were calculated to define farm characteristics. Kruskal-Wallis test was used to investigate differences in number of sows and farm type. To investigate associations between management factors and antibiotic use and resistance, univariable logistic regression and Fisher’s exact test were used. The statistical significance level was defined as a two-tailed P-value ≤ 0.05.

RESULTS

Farm locations

The farms were located in a radius of ca. 20 kilometers from Khon Kaen City in Khon Kaen Province, north-eastern Thailand. The locations can be seen in figure 2.

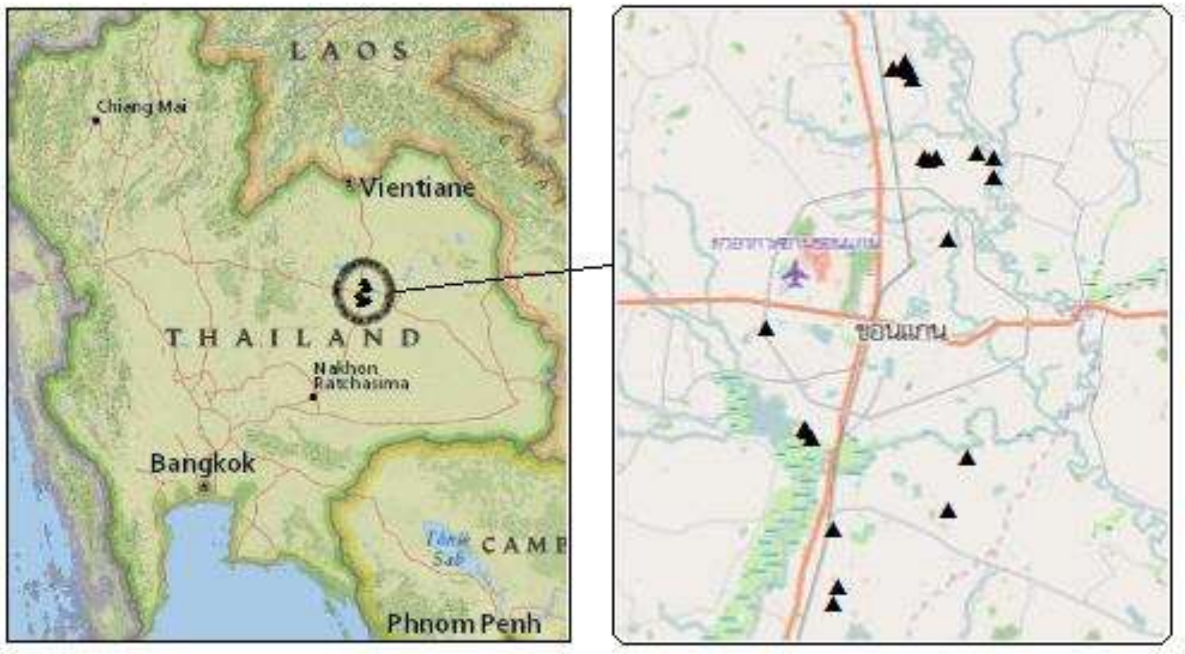


Figure 2: *Shows the locations of the farms visited in Khon Kaen Province. Photo: Gunilla Ström*

Farm characteristics and antibiotic use

Housing

The most common type of farm (60%) was the "farrow-to-finish" type, i.e. a farm that raised pigs from farrowing until they are ready for slaughter. A lesser number of farms (28%) were identified as breeding farms and sold their piglets after weaning, while only a small proportion of farms combined these practices (Table 1).

Table 1: *The different types of farms*

Type of farm	%	n=25
Farrow-to finish	60%	15
Breedning	28%	7
Combination of the two	12%	3

The number of sows on the farms varied from one to nineteen, with a median of five sows (5 and 95% percentiles: 1.2 and 11.8) per farm. The median number of sows on farrow-to-finish farms was four, and for breeding farms ten, but the difference was not statistically significant.

The farms acquired the sows either through breeding their own, or by buying from other farms (table 2). Some farms acquired their sows both by breeding their own and buying from other farms. Farms that kept more sows were more likely to breed their own (P = 0.019).

Table 2: *The different ways the farms acquired the sows*

Where do you get your sows?	**%**	**n=25**
Breed your own	28%	7
From another farm in the district	32%	8
From another farm outside the district	16%	4
Breed your own and buy from another farm in the district	16%	4
Breed your own and buy from another farm outside the district	8%	2

There were no free-roaming pigs of any age group on any of the farms (n=25). Different means of confining the sows were used and are shown in table 3. The enclosures ranged from conventional metal crates that restricted the sows' ability to move except for lying down or standing up, to larger pens where the sows were free to move around. Which type of confinement that was used, differed somewhat between nursing and gestation sows, in that nursing sows were more likely to be fully confined in a metal crate than the gestation sows.

Table 3: *The different means of confining the sows*

Nursing sows confinement	**%**	**n=24**	**Gestation sow confinement**	**%**	**n=22**
small metal crate	50.0%	12	small metal crate	36.4%	8
pen ≤3x3m	45.8%	11	pen ≤3x3m	59.1%	13
pen >3x3m	4.2%	1	pen >3x3m	4.5%	1

No types of cooling systems were used on the farms, instead all of the farms (n=25) used so called open-air systems, which allows the wind to blow through the enclosures. This also allows for birds and other animals to enter the enclosures and come in contact with the pigs. All the farms (n=24) kept the sows on floors that were made of solid concrete except one that used dirt floors. Finally, all the farms (n=25) practiced a "continuous flow system" of sows in the farrowing units.

Management and antibiotic usage

The cleaning intervals at the farms are presented in table 4. About 50% of the farms reported that they clean the floors daily.

Table 4: *The cleaning intervals in the pens*

How often are faeces removed from the floor?	%	n=25
1/day	24%	6
2/day	28%	7
1-4 times per week	12%	3
More seldom than once per week	0%	0
Never	36%	9

All the farms reported that they vaccinated their sows, but only 17 of the farms were able to tell what diseases they vaccinated against. All of these farms vaccinated against classical swine fever. Table 5 shows the list of vaccines used and how commonly used these were.

Table 5. *The table shows the number and proportion of farms that used different vaccines (n=17)*

Vaccine	Number of farms	Percentage
Classical swine fever	17	100.0%
Erysipelothrix/parvo combination	5	29.4%
Mycoplasma	5	29.4%
Rabies	8	47.1%
PRRS	3	17.7%
Aujeszky's disease	2	11.8%

Antibiotics for injection were reportedly used on all the farms, although only 21 of the farms were able to tell what kinds of antibiotics they used (Table 6). The most common kind used for injection was enrofloxacin, which was used on 44% of the farms. Amoxicillin was used on 32% of the farms, followed by kanamycin which was used on 20% of the farms.

Only one farm reported using antibiotics in the feed to treat sick animals. Amoxicillin, sulfonamide and oxytetracycline were used, but the farmer was unable to specify the amounts that were used.

Table 6. *The table shows the number and proportion of farms that uses a specific antibiotic (n= 21)*

Antibiotic	Number of farms	Percentage
Amoxicillin	8	32%
Enrofloxacin	11	44%
Penicillin/streptomycin combination	4	16%
Kanamycin	5	20%
Florfenicol	1	4%
Oxytetracycline	1	4%
Gentamicin	1	4%

Eight farms reported using antibiotics as a routine after farrowing. The sow would get one or more injection to prevent illness during the nursing period. This was not a question included in the questionnaire however, so there is no data regarding the farms that did not specify this on their own initiative.

When it came to deciding about how and when to give antibiotics to the sows, the most common way was for the farmer to decide (see table 7). This was reportedly the case in 64% of the farms, while 32% reported that a veterinarian decided. One farm reported enlisting the help of the owner of a neighboring farm to decide about when to give antibiotics.

There was no significant difference in the resistance pattern depending on whether the owner or veterinarian decided about the antibiotic treatments.

Table 7: *Shows who decides about the antibiotic treatments*

Who decides about when and how to give antibiotics if a sow gets ill?	**%**	**n=25**
Veterinarian	32%	8
Owner of the farm	64%	16
Other person	4%	1

The most common way to get access to antibiotics was to buy it from a local store or pharmacy, with 60% of the farms acquiring their antibiotics this way. The second most common method was to buy directly from a veterinarian, which 32% of the farms did (Table 8).

There was no significant difference in the resistance pattern depending on whether the farmer bought the antibiotics in a local store/pharmacy or directly from the veterinarian.

Table 8: *Shows the different ways farms got access to antibiotics*

How do you get access to the antibiotics/where do you buy it?	**%**	**n=25**
Buy from veterinarian	32%	8
Buy from local store/pharmacy	60%	15
Buy from contract farm	4%	1
Buy from other place	4%	1

The majority of the farmers (60%) reported that they treat a sow on the farm two to three times per year on average (table 9). Only 15% reported that they treat a sow more often than three times per year.

Table 9: *Shows how often a sow was treated on the farm*

How often on average per year is a sow treated intramuscularly?	%	n=20
1 /year	25%	5
2-3/year	60%	12
>3/year	15%	3

Laboratory results - antimicrobial susceptibility

Escherichia coli was successfully isolated from all 69 samples. The distribution of MICs and percent resistance are shown in table 14. To see the individual resistance pattern for each farm, see table 15.

All of the farms produced an isolate that showed resistance against at least one type of antibiotic (Table 15). Resistance against tetracycline (75.3% of the isolates), ampicillin (69.6%) and sulfamethoxazole (60.8%) were the most common, followed by ciprofloxacin (40.5%) and chloramphenicol (39.1%)

Multiresistance could be seen in 69.5% of the isolates, while 10.1% of the isolates were susceptible to all types of antibiotics (Table 10).

Table 10. *Quantity and proportion of isolates resistant to none or all of the tested antibiotic classes*

Resistance	No. of isolates	Percentage
Susceptible to all	7	10.1%
Resistant against 1-2 classes	14	20.3%
Resistant against 3-5 classes	39	56.5%
Resistant against >5 classes	9	13.0%

Of the multiresistant isolates, 63.3% shared resistance to the three most common antibiotics tetracycline, ampicillin and sulfamethoxazole. This and the individual isolates proportion of resistance against these antibiotics are shown in table 11.

Table 11. *Quantity and proportion of the multiresistant isolates (n=48) resistant to ampicillin, tetracycline and sulfamethoxazole*

Antibiotics	No. of isolates	Percentage
Am	45	91.8%
Tc	44	89.8%
Su	40	81.6%
Am + Tc + Su	31	63.3%

One isolate showed the characteristics of a possible ESBL-producing bacterium in that it showed resistance to ampicillin, cefotaxime and ceftazidime. The MICs are shown in table 12.

Table 12. *The MICs (mg/L) for the suspected ESBL-producing isolate*

Antibiotics	**Am**	**Ci**	**Nal**	**Gm**	**Sm**	**Tc**	**Ff**	**Cs**	**Su**	**Trim**	**Cm**	**Mp**	**Ctx**	**Caz**
Suspected ESBL isolate	>128	>1	>128	1	8	64	8	2	≤8	0.5	>32	0.06	>2	>4

In some cases, farms that showed resistance against one type of antibiotic had a tendency to also show resistance against another type (Table 13).

Table 13: *Farms resistant to antibiotic 1 had a tendency to also show resistance to antibiotic 2*

Antibiotic 1	**Antibiotic 2**	***P*-value**
Sulfamethoxazole	Trimetoprim	$P = 0.057$
Ciprofloxacin	Sulfamethoxazole	$P = 0.024$
Ciprofloxacin	Trimethoprim	$P = 0.081$
Ampicillin	Tetracycline	$P = 0.120$

Table 14: *Resistance and distributions of MIC for the bacteria tested (n=69). Vertical lines mark the ECOFFs. White fields denote range of dilutions tested for each substance. MICs above the tested range are shown as the concentration closest above the tested range (in the blue field). MICs equal to or lower than the lowest concentration tested are shown as the lowest tested concentration*

Antimicrobial agent	**Resistance** (%)	**Distributions (%) of MICs (mg/L)** ≤0.008	**0.016**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**>1024**
Ampicillin	69.6								8.7	20.3	1.4	▌				2.9	66.7			
Ciprofloxacin	40.5		1.4	18.8	39.1▌	2.9	5.8	10.1	8.7	13.0										
Nalidixic acid	20.2									8.7	46.4	20.3	4.3▌	7.2		2.9	10.1			
Gentamicin	11.4						7.2	36.2	34.8	10.1▌	4.3		1.4	1.4	4.3					
Streptomycin	37.6									7.2	17.4	21.7	15.9▌	10.1	1.4	14.5	2.9	8.7		
Tetracycline	75.3								2.9	11.6	7.2	2.9▌	8.7	30.4	30.4	5.8				
Florfenicol	14.5										5.8	62.3	17.4▌		14.5					
Colistine	0.0							73.9	14.5	11.6▌										
Sulfamethoxazole	60.8											26.1	10.1	2.9	▌			1.4		59.4
Trimethoprim	42.0						4.3	36.2	15.9	1.4▌				42.0						
Chloramphenicol	39.1										20.3	37.7	2.9▌	21.7	17.4					
Cefotaxime	1.4			15.9	66.7	14.5	1.4▌				1.4									
Ceftazidime	4.3					13.0	66.7	15.9▌	2.9			1.4								

Table 15: *The individual resistance patterns for the farms. Each letter represents one isolate*

Farm No.	Am	Ci	Nal	Gm	Sm	Tc	Ff	Cs	Su	Trim	Cm	Ctx	Caz
1	RSS	RSS	SSS	SSS	SSS	RRR	SSS	SSS	RSR	SSR	SSS	SSS	SSS
2	SSS	RRS	RSS	RSS	SSS	RSS	RSS	SSS	RSS	RSS	RSS	SSS	SSS
3	RSS	RSS	SSS	SSS	RSS	RRR	RSS	SSS	RRS	SRS	RRS	SSS	SSS
4	RRS	RRS	RRS	SRS	RSS	RRR	RSS	SSS	RRR	RSR	RSS	SSS	SSS
5	RRR	SRS	SSS	SSS	SSR	RRR	SSS	SSS	RRR	RSR	SSR	SSS	SSS
6	RRR	SRR	SSS	SSS	RSS	SRR	RSS	SSS	RRR	RSR	RSR	SSS	SSS
7	RRR	RRR	RRS	RSS	RRR	RSR	SSS	SSS	RRS	RSS	RSS	SSS	SSS
8	SRR	SRR	SRS	SSS	SRS	SRR	SSS	SSS	SRR	SRR	SRR	SSS	SSS
9	RRR	SSS	SSS	SSS	SRS	RRR	SSS	SSS	RSS	SSS	SSS	SSS	SSS
10	RRR	RSS	RSS	SSS	SSS	SSR	SSS	SSS	SRR	SSR	SRR	SSS	SSS
11	SSS	SSS	SSS	SSS	SRR	SSS	SSS	SSS	SSS	SSR	SSS	SSS	SSS
12	RRR	SSS	SSS	SSS	RSS	RRR	SSR	SSS	RRS	RRS	SRR	SSS	SSS
13	R	R	S	S	R	R	S	S	R	R	R	S	S
14	RSR	SSR	SSS	SSS	RSS	RRR	SSS	SSS	SSR	SSS	SSS	SSS	SSR
15	RR	SR	SR	SS	RS	RR	SS	SSS	RR	RR	SS	SS	SS
16	RRS	SSS	SSS	SRS	SSS	SRS	SRS	SSS	SSS	SSS	SRS	SSS	SSS
17	RSS	SSS	SSS	SSS	SRS	RRR	SSS	SSS	RRS	RRS	RRS	SSS	SSS
18	RRR	RRR	SSS	SSS	SSR	RRR	SSS	SSS	RSR	RSS	RSS	SSS	SSS
19	RRS	SSS	SSS	SRS	RRS	SRS	SSS	SSS	RSS	RSS	SSS	SSS	SSS
20	RSR	RSR	RSS	SSR	RSR	RSR	SSS	SSS	RSR	SSR	SSR	SSS	SSS
21	RRR	RRR	RRR	RSS	RRR	RRR	RSS	SSS	RRR	RRS	RSR	SSS	SSS
22	R	R	R	R	S	R	R	SSS	R	R	R	S	S
23	RRR	RRS	SRS	SSS	RSR	RRR	SSR	SSS	RSR	SSR	RRR	SRS	SRS
24	SRR	SSS	SSS	SSS	SSS	RRS	SRS	SSS	RSR	RSS	SRS	SSS	SSR
25	SS	SS	SS	SS	SS	SR	SS	SSS	SS	SS	SS	SS	SS

🟧	Every sample from the farm were resistant against this type of antibiotic
🟩	Every sample from the farm were susceptible against this type of antibiotic
▨	The samples from the farm were different in their resistance pattern

DISCUSSION

The results indicate that AMR is common in small-scale pig farms in north-eastern Thailand. There may be many different reasons for this, of which some are discussed below.

Although only one farm (4%) reported treating the sows with oxytetracycline, as many as 75.3% of the isolates showed resistance against tetracycline. The same can also be seen when looking at sulfamethoxazole and trimethoprim, where no farms reported any usage of these antibiotics but the resistance rates were 60.9 and 42.0% respectively. A reason for this could perhaps be that these antibiotics were formerly used in great volumes in pig farms throughout the area and have become obsolete due to the fact that they are now less useful because of resistance against the drugs. The resistance mechanisms may still be present though, which could explain this resistance pattern. Another explanation could be that resistance is present due to co-selection, where the usage of one type of antibiotic facilitates the development of resistance against other types.

A similarly high resistance rate can be seen in ampicillin (69.6% of the isolates). Amoxicillin, which is in the same family of antibiotics as ampicillin, is however still in use in 32% of the farms. It would be interesting to perform a study similar to this one within a couple of years to see if the usage of amoxicillin has changed.

A majority of the farms acquired their antibiotics from local stores and decided themselves when to treat the animals without any consultation from a veterinarian. This could perhaps be one reason why the resistance rate is higher in Thailand than in some of the OECD countries where antimicrobial drugs require a prescription from a veterinarian or are regulated in more strict ways (table 17). The ease of which these drugs are acquired might increase the consumption and thereby promote the development of AMR.

Additional factors that might increase the need for and use of antimicrobial drugs are the housing system and flow system of the farms. All farms utilized an open air system and a continuous flow system in the farrowing units. The open air system allows the wind to blow through the enclosures. This also allows birds and other animals, but also pathogens to come in contact with the pigs. A continuous flow system in the farrowing units makes adequate cleaning and disinfection difficult and facilitates disease spread between animals since sows continuously are mixed together. The hot and humid climate in Thailand also increases the risks for bacterial and fungal diseases to spread. All of this may result in increased usage of antimicrobial drugs.

The results from seven studies on AMR from different countries throughout the world are presented in table 17 together with the results from this study. These studies show some differences in the antimicrobial resistance pattern. However, one must not compare the data from these studies without taking into consideration the differences in methodology from the different studies.

The studies from Sweden (SVARM, 2012), Europe (ECDC, 2014), Denmark (DANMAP, 2015) and Halje (forthcoming) are comparable to this one, due to the fact that they all follow the standardized methodology of antimicrobial susceptibility testing (European Committee for Standardization, 2007) and ECOFFs provided by EUCAST to interpret the results. Worth noting is that the results from EFSA & ECDC (2015) are the combined results from ten different member states. The level of resistance between different member states may differ.

The Canadian results (CIPARS) must be interpreted with the knowledge that the breakpoints used to determine resistance were in general higher than the ECOFFs (CIPARS, 2015a). This means that an isolate classified as resistant in this study, might not be so in CIPARS. Therefore the percentage of resistance might be higher than presented in table 17.

Likewise, one must interpret the results from the two Thai studies (Jiwakanon *et al., 2008;* Boonyasiri *et al., 2014*) with caution as there is no information regarding the methods used to interpret the AMR data.

Even so, there are remarkable differences in the antimicrobial resistance patterns. Sweden had the lowest rate of resistance in virtually all the tested antibiotics except chloramphenicol, ciprofloxacin and nalidixic acid, where Denmark had a lower resistance rate (Table 17). The resistance rates were generally higher in all the Thai studies than in the rest of the studies (Table 17). Likewise, the levels of multiresistance in this report and Halje (forthcoming) (69.5 and 95.1%, respectively) were higher than what was reported in Sweden (13%) (Table 16).

Table 16: *Resistance to different numbers of antibiotic classes. Numbers taken from SVARM, 2012; Halje (forthcoming) and this report*

Area (Numbers of isolates tested)	**Percentage of isolates resistant to different numbers of antibiotic classes**			
	0	**1**	**2**	**≥3**
Sweden (167)	72.0	9.0	5.0	13.0
Thailand 2015 (Halje) (81)	2.5	0.0	2.5	95.1
Thailand 2015 (Karlsson) (69)	10.1	11.6	8.7	69.5

When comparing this study and the Halje study (small-scale farms vs medium-scale industrial farms in Thailand), the level of resistance was higher in all types of antibiotics in the Halje study, except for gentamicin and Cefotaxime. This could possibly be because of the differences in antibiotic usage between the two types of farms. It is reasonable to believe that the antibiotic usage on medium-scale industrial farms is more organized and continuous than it is in smaller backyard farms.

Boonyasiri *et al.* (2014) reported on high levels of ESBL-producing *E. coli;* 80.2% from the northern Thai province and 64.7% from the eastern province. This is in contrast to the findings in this study, where only one possible ESBL-isolate was found, and the rest of the studies. Here as well it is reasonable to believe that the unregulated usage of antibiotics in Thai farms is the factor behind these findings. A possible reason for the big difference in the occurrence of ESBL-producing *E. coli* between the Boonyasiri study and this one is that the methods differed. The former used agar plates containing cephalosporins when isolating the bacteria, which selects for the growth of ESBL-producing bacteria. This makes the comparison between the two studies impossible when it comes to the occurrence of ESBL-producing *E. coli*.

There is a difference in the usage of antibiotic drugs in livestock between the different countries and this might reflect the resistance pattern. Since Sweden has very strict regulations and practices on the use of antibiotic drugs and Thailand a loosely regulated one, it is maybe not that difficult to accept that Sweden has the lowest resistance rate and Thailand the highest.

Table 17: *Antimicrobial susceptibility in pigs in Canada, Europe, Denmark, Sweden and Thailand. Numbers taken from CIPARS,2015c*; EFSA & ECDC, 2015***; DANMAP, 2015; SVARM,2012; Jiwakanon et al., 2008**; Boonyasiri et al.,2014**; Halje (forthcoming) and this report*

Percent of isolate resistant from different countries.

Area (number of isolates tested)	**Aminoglycosides**				**Penicillins**	**Cephalosporines**						**Folate pathway inhibitors**		
	Gm	**Km**	**Sm**	**Ak**	**Am**	**Ctr**	**Cox**	**Cef**	**Cti**	**Ctx**	**Caz**	**TrSu**	**Tm**	**Sua**
Canada, 2013 (1573)	1.0	12.5	34.0		31.1	1.4	1.1		1.1			13.4		45.4
Europe, 2013 (1954)	1.8		47.8		30.3					1.3				42.1
Denmark, 2013 (209)	1.0				33.0					0.0	0.0		24.0	34.0
Sweden, 2011 (167)	1.0	1.0	16.0		13.0					<1.0			11.0	17.0
Thailand, 2008 (338)	30.8	40.8	66.3		84.5			2.0		0.5		85.2	87.3	
Thailand, 2014 North (330)	58.6			0.6		59.7	2.4							
Thailand, 2014 East (70)	55.4			12.9		57.1	35.7							
Thailand, 2015 (Halje) (81)	7.4		76.5		85.2					1.2	3.7		70.4	84.0
Thailand, 2015 (Karlsson) (69)	11.4		37.6		69.6					1.4	4.3		42.0	60.8

** The breakpoints used in CIPARS are in general higher than the ECOFFs. Thus, the level of resistance might in fact be higher than shown here, if one were to use ECOFFs instead.*

***These results must be interpreted with caution, since there is no information regarding the methods used to interpret the AMR data.*

****The resistance level presented here is the combined results from 10 different member states. The individual level of resistance differs between countries.*

Area (number of isolates tested)	**Amphenicols**		**Polymyxins**	**Quinolones**		**Tetracycline**
	Cm	**Ff**	**Cs**	**Ci**	**Nal**	**Tc**
Canada, 2013 (1573)	20.3				0.3	75.4
Europe, 2013 (1954)	14.7			6.1	3.8	52.8
Denmark, 2013 (209)	2.0		0.0	0.0	0.0	37
Sweden, 2011 (167)	4.0	0.0	0.0	2.0	2.0	8.0
Thailand, 2008 (338)			3.5	26.8	37.6	97.9
Thailand, 2014 North (330)			0.3–2.9	32.7	12.1	62.4
Thailand. 2014 East (70)			0.3–2.9	71.4	51.4	84.3
Thailand, 2015 (Halje) (81)	58.0	2.4	0.0	48.1	30.8	86.3
Thailand, 2015 (Karlsson) (69)	39.1	14.5	0.0	40.5	20.0	75.3

Antibiotics:

Gm	Gantamicin	**TrSu**	Trimethoprim-sulfamethoxazole
Km	Kanamycin	**Tm**	Trimethoprim
Sm	Streptomycin	**Sua**	Sulfonamides
Ak	Amikacid	**Cm**	Chloramphenicol
Am	Ampicillin	**Ff**	Florfenicol
Ctr	Ceftriaxone	**Cs**	Colistin
Cox	Cefoxitin	**Ci**	Ciprofloxacin
Cef	Cefuroxine	**Nal**	Nalidixic acid
Cti	Ceftiofur	**Tc**	Tetracycline
Ctx	Cefotaxime		
Caz	Ceftazidime		

To find suitable farms sometimes proved to be difficult due to uncertain information about the number of sows and precise geographical location of the farms. In addition, poor communication options made direct contact with the farmers difficult. The translator often had to make contact with the head of the village, who then provided the information needed to find the farms.

In Decision 2003/652/EU there are instructions on the sampling strategy for the harmonised AMR monitoring in EU. These instructions specify that samples are to be taken from fattening pigs, since they are closest to the consumer in the food chain (EFSA, 2014). The decision to collect samples from sows instead of fattening pigs was because at small-scale farms there might not always be fattening pigs available for sampling.

The answers from question number 10b “How often are the floors washed with water/soap/disinfection?” (Appendix 1) were excluded from this report, due to the fact that the question was badly phrased. The answers given did not differentiate between cleaning the floors using only water, using only disinfectant or a combination of the two, as was intended when asking the question.

Statistical analyses were made to find possible risk factors for AMR and investigate other associations between management factors, antibiotic use and AMR. However, since only 25 farms were visited, these statistical analyses were difficult to perform due to the small number of observations and those made must therefore be considered to lack in statistical power.

CONCLUSIONS

Antimicrobial resistance in small-scale swineherds in north-eastern Thailand seems to be common. Even though a number of studies have been done on the subject, there is still a lot of uncertainty on the full width of the problem. A standardized and harmonized program similar to the ones in Europe and Canada is needed to map AMR throughout the country and be able to compare the data with other countries. A program like this is currently in development by the Thai DLD and will hopefully be active soon. Furthermore the usage of antimicrobial drugs in the country must be surveyed and regulated to avoid the problem with AMR to grow even further.

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Appendix 1

Questionnaire – small farms

Name of the owner of the farm:	GPS-coordinations:
Working position of person answering the questionnaire:	Type of farm (farrow-to-finish/breeding/other)
Number of sows:	Number of weaned pigs:

1. From where do you get your sows?

a. Breed our own
b. From another farm in the district
c. From another farm, not in the same district
d. Other:

Questions about antibiotic usage

2. Do you give antibiotics in the feed?

Yes/No

3. What kind of antibiotics do you use?

a. In the daily feed for nursing sows:

b. In the daily feed for gestation sows:

c. For injections in sows that are ill (including treatment protocol):

Appendix 1

Type of antibiotic *Number of days in treatment*

d. By feed to sows that are ill (including treatment protocol):

Type of antibiotic *Number of days in treatment*

4. At how many occasions (on avarage) per year is a sow treated (p.o. respectively i.m.)

Per os :

Intramuscular :

5. How do you get access to the antibiotics? Where do you buy it?

a. Buy from veterinarian

b. Buy from local store/pharmacy

c. Buy from contract farms

d. Other:

6. *How much feed* do the sows get per day and *how much antibiotics* does the feed contain?

	How often?	How much?	Antibiotic concentration?
Nursing	Times/d	Kg/d	
Gestation	Times/d	Kg/d	

Appendix 1

7. Who decides about when and how to give antibiotics if a sow gets ill?

a. Veterinarian
b. Small doctor/technician
c. Owner of the farm
d. Worker on the farm
e. Other person:

8. Do you vaccinate the pigs? Against which diseases?

Yes/No

Questions regarding husbandry

9. Do you use a *"continuous flow system"* or an *"all-in, all-out system"* in the farrowing units?

10. How often are the floors

a. cleaned from faeces with a broom or something similar?

b. washed with water/soap/disinfection?

a.

b.

11. What type of disinfection do you use for cleaning?

Appendix 1

For us to fill in

12. Type of cooling system? (Evaporation/conventional (open air) system)

    a. Evaporation

    b. Conventional (open air)

    c. Other:

13. What type of floor do the sows have?

14. Are the sows confined? What type of confinement?

15. Density of farms in the village? (For us, look at the map)