



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine
and Animal Science**
Department of Clinical Science

Antimicrobial resistance in indicator *Escherichia coli* from medium-sized swine herds in North-eastern Thailand

Matilda Halje

*Uppsala
2016*

Degree Project 30 credits within the Veterinary Medicine Programme

*ISSN 1652-8697
Examensarbete 2016:61*

Antimicrobial resistance in indicator *Escherichia coli* from medium-sized swine herds in North-eastern Thailand

Antimikrobiell resistens hos indikatorbakterie *Escherichia coli* på medelstora svinbesättningar i nordöstra Thailand

Matilda Halje

Supervisor: Ulf Magnusson, Department of Clinical Sciences

Assistant Supervisor: Märit Pringle, SVA

Examiner: Johanna Lindahl, Department of Clinical Sciences

Degree Project in Veterinary Medicine

Credits: 30

Level: Second cycle, A2E

Course code: EX0736

Place of publication: Uppsala

Year of publication: 2016

Number of part of series: Examensarbete 2016:61

ISSN: 1652-8697

Online publication: <http://stud.epsilon.slu.se>

Key words: Antibiotic resistance, pigs, Thailand

Nyckelord: Antibiotikaresistens, gris, Thailand (key words in Swedish)

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

SUMMARY

Antimicrobial resistance (AMR) is a fast growing global threat in several perspectives. In medicine the antimicrobials are crucial in the treatment of some diseases and without the antimicrobials those diseases might be fatal. In veterinary medicine antimicrobials are used as treatment, prevention and growth promoters and without them it would be difficult to handle some diseases which could result in extensive economic losses for the animal owner as well as for the society as a whole, especially in developing countries.

Excessive and imprudent use of antibiotics may contribute to the development and dissemination of resistant bacteria and genes. Several studies have shown a risk for dissemination of resistant bacteria from food animals to humans and therefore measures have been taken on national as well as on international levels to curb this progression. One example of such measures is surveillance systems to monitor the resistance pattern of selected microbes regularly. A gained knowledge about the resistance patterns, along with knowledge about resistance mechanisms, makes it possible to adjust regulations and recommendations for antibiotic usage so that less broad-spectrum antibiotics are used in favor for the narrow-spectrum antibiotics or, for that matter, no antibiotics at all.

Improvements in preventive management such as good hygiene and biosecurity would also decrease the need for antimicrobials in animals and livestock which would be beneficial in hindering the progression of AMR.

This study aims to contribute to the important monitoring and mapping of AMR in livestock. The pig production in Thailand is expanding and an increasing number of large-scaled farms are appearing at the same time as the number of smaller farms decreases. Therefore Thailand was chosen for this study.

In this study indicator *Escherichia coli* was cultured from rectal swabs from healthy sows on 27 medium-sized (100-500 sows) farms in the northeast of Thailand. Samples were collected from three sows at each farm, resulting in 81 samples in total. To test them for antibiotic susceptibility a VetMIC GN-mo panel was used – a MIC-based (minimum inhibitory concentration) broth-microdilution method. Antibiotic substances included in the study were: amoxicillin, ciprofloxacin, nalidixic acid, gentamicin, streptomycin, tetracycline, florfenicol, colistin, sulfamethoxazole, trimethoprim, chloramphenicol, meropenem, cefotaxime and ceftazidime.

At each farm a questionnaire was also filled in to enable identification of possible risk factors for antibiotic resistance. The questions were chosen and formulated in a manner that would give us insight in the routines regarding antibiotic usage, husbandry and health status of the pigs.

From 81 samples, 81 *Escherichia coli* isolates were obtained. The percentage of resistant isolates among the tested isolates for each of the included antibiotics was as follows: ampicillin (85.2%), ciprofloxacin (48.1%), nalidixic acid (30.8%), gentamicin (7.4%), streptomycin (76.5%), tetracycline (86.3%), florfenicol (2.4%), colistin (0.0%), sulfamethoxazole (84.0%), trimethoprim (70.4%), chloramphenicol (58.0%), cefotaxime (1.2%) and ceftazidime (3.7%). Multidrug resistance (MDR) was found in 95.1% of the isolates. The variations in management

and antibiotic usage among the farms were very small and therefore statistical relationships could not be obtained in regards to management, antibiotic usage and antibiotic resistance.

Some of the results for meropenem were found to be unreliable. One of the strains (M13) had nevertheless a high minimum inhibitory concentration (MIC) for meropenem as well as for other betalactams and is therefore possibly ESBL_{CARBA}-producing (extended spectrum betalactamase- and carbapenemase-producing). Such finding would be perturbing since an ESBL_{CARBA}-producing strain are resistant to several highly important antimicrobials. This result needs however to be further investigated with PCR (polymerase chain reaction).

Although there are undertakings regarding AMR in Thailand, the usage of antimicrobials in animals remains less defined and the presence of AMR seems to be high compared to Sweden and Europe as well as Canada. An AMR surveillance program is necessary in Thailand as well as other Southeast Asian countries to be able to draw plausible conclusions regarding the AMR and the effect of antibiotic usage in this region.

This study shows a wide use of antibiotics in the farms included. All of the farms administered antibiotics to the sows as injection as a routine after farrowing. The results from the antibiotic susceptibility tests display a generally high resistance frequency for a majority of the included antibiotics. This indicates that a wide use of antibiotics results in resistant bacteria, which makes a prudent antibiotic use, as well as surveillance systems, crucial to curb the development of more resistant bacteria.

SAMMANFATTNING

Antimikrobiell resistens (AMR) är ett globalt växande problem ur såväl humanmedicinska, veterinärmedicinska och samhällsekonomiska perspektiv. Antimikrobiella läkemedel är avgörande för behandlingen av vissa sjukdomar och utan rätt behandling kan dessa sjukdomar innebära dödlig utgång. Inom veterinärmedicin används antimikrobiella medel i behandlingen av sjukdomar såväl som i förebyggande och tillväxtfrämjande syfte. Brist på fungerande antimikrobiella medel kan därför resultera i omfattande ekonomiska förluster för djurägare såväl som för hela samhället, speciellt i utvecklingsländer.

Den utbredda och ansvarslösa användningen av antibiotika bidrar till utvecklingen och spridningen av resistenta bakterier och resistensgener. Flera studier har påvisat en risk för spridning av resistenta bakterier från produktionsdjur till människa, därför har åtgärder vidtagits på nationell- och internationell nivå för att motverka denna utveckling. Exempel på åtgärder är de övervakningssystem som finns i många länder för att regelbundet se över resistensmönster av utvalda mikrober. Med ökad kunskap om resistens samt om mekanismerna bakom resistensutvecklingen är det möjligt att förändra regler och rekommendationer för antibiotikaanvändning så att antibiotika med brett spektra ersätts av de med smalare spektra, alternativt att antibiotika inte används alls i de fall det inte behövs.

Förbättringar i förebyggande åtgärder så som god hygien och ökad biosäkerhet skulle minska behovet av antimikrobiella medel till produktionsdjur, vilket också skulle bidra till förbättring av resistensläget.

Denna studie ämnar bidra till den så viktiga övervakningen och kartläggningen av AMR hos livsmedelsproducerande djur. Grisproduktionen i Thailand är omfattande och mängden gårdar med storskalig grisproduktion ökar kontinuerligt, därför var Thailand ett lämpligt val för studien.

I denna studie isolerades indikatorbakterier av arten *Escherichia coli* från rektalsvabbar från friska suggor på 27 medelstora (100-500 suggor) grsigårdar i nordöstra Thailand. Prover togs från tre suggor på varje gård vilket resulterade i totalt 81 prover. Känslighetstestet utfördes med hjälp av VetMIC (panel GN-mo), vilket är en MIC-baserad (minimum inhibitory concentration=minsta hämmande koncentration) buljong-mikrodilutionsmetod. Följande 14 antibiotikasubstanser testades i studien: amoxicillin, ciprofloxacin, nalidixinsyra, gentamicin, streptomycin, tetracyklin, florfenikol, kolistin, sulfametoxazol, trimetoprim, kloramfenikol, meropenem, cefotaxim och ceftazidim.

På varje gård besvarades ett frågeformulär för att identifiera möjliga riskfaktorer för antimikrobiell resistens. Frågorna var utvalda och formulerade på ett sätt som kunde ge insyn i rutinerna i antibiotikaanvändning, skötsel och hälsostatus hos grisarna.

Escherichia coli kunde isoleras från alla 81 prover. Andelen resistens för respektive antibiotikum var följande: ampicillin (85,2 %), ciprofloxacin (48,1 %), nalidixinsyra (30,8 %), gentamicin (7,4 %), streptomycin (76,5 %), tetracyklin (86,3 %), florfenikol (2,4 %), kolistin (0,0 %), sulfametoxazol (84,0 %), trimetoprim (70,4 %), kloramfenikol (58,0 %), cefotaxim (1,2 %) och ceftazidim (3,7 %). Multiresistens (MDR) observerades hos 95,1 % av alla isolat. Variationerna i skötsel, hälsostatus och antibiotikaanvändning mellan gårdarna var mycket små och därför kunde inte statistiska samband observeras mellan dessa faktorer och antibiotikaresistensen.

En del av resultaten för meropenem konstaterades vara opålitliga och togs därför bort ifrån studien. En av stammarna (M13) hade dock ett högt MIC-värde för både meropenem och de andra betalaktamerna vilket innebär att den skulle kunna vara ESBL_{CARBA}-producerande. Ett sådant fynd är oroande eftersom en ESBL_{CARBA}-producerande stam är resistent mot flera av våra viktigaste antibiotika. Detta resultat behöver emellertid undersökas vidare med polymerase chain reaction (PCR).

Trots vidtagna åtgärder gällande antimikrobiell resistens i Thailand råder oklarheter i användandet av antimikrobiella medel till djur. Man ser också att förekomsten av resistent bakteriestammar förefaller vara vanligare än i såväl Sverige och Europa som Kanada. Ett övervakningsprogram är nödvändigt i Thailand och andra Sydostasiatiska länder för att kunna dra några slutsatser rörande den antimikrobiella resistensen och effekten av antibiotikaanvändningen i denna region.

Denna studie visar en utbredd antibiotikaanvändning på de inkluderade gårdarna. Alla gårdar hade som rutin att ge en antibiotikainjektion efter grisning. Resultaten från resistenstesten visade en generellt hög resistensförekomst mot majoriteten av inkluderade antibiotika. Detta indikerar att en utbredd antibiotikaanvändning kan resultera i resistent bakterier. En ansvarsfull användning, såväl som övervakningssystem, är således nödvändigt för att bromsa utvecklingen av fler resistent bakterier.

INDEX

Abbreviations	11
Abbreviations for antibiotic substances.....	11
Introduction	13
Antimicrobial resistance.....	13
Objectives of field work.....	14
Multidrug resistance.....	15
Literature Study.....	16
Pig industry in Thailand	16
Reproductive performance and diseases in Thailand	16
Surveillance of antibiotic usage in animals and livestock and antimicrobial resistance in Thailand	17
Antibiotic usage and surveillance in pigs in Sweden	19
Antibiotic susceptibility in Canada, Europe, Sweden and Thailand	19
Studies on ESBL and ESBL _{CARBA}	23
Material and methods	23
Field sampling.....	23
Isolates.....	24
Antimicrobial susceptibility testing.....	24
Cut-off values.....	24
Questionnaire	25
Statistical analysis	25
Results	26
Farm management and veterinary service.....	26
Location.....	27
Antibiotic usage.....	28
Antibiotic resistance.....	29
Statistical analysis	Fel! Bokmärket är inte definierat.
Discussion	34
Associations between antibiotic resistance, antibiotic usage and management factors	34
Statistical analysis	34
ESBL and ESBL _{CARBA}	34
Comparison with data from Sweden, Europe and North America	35
Antimicrobial usage	35
Antimicrobial susceptibility	35
Conclusions	36

Acknowledgements	37
References	38
Appendices	i
Appendix 1	i
Appendix 2	ii

ABBREVIATIONS

AD – Aujeszky's disease

AMR – Antimicrobial resistance

AR – Atrophic rhinitis

CAMHB – Cation-adjusted Mueller Hinton Broth

CSF – Classical swine fever

E. coli – *Escherichia coli*

EUCAST – European Committee on Antimicrobial Susceptibility Testing

FAO – Food and Agriculture Organization of the United Nations

FMD – Foot-and-mouth disease

GPA – Growth Promoting Antibiotics

MDR – Multidrug Resistance

MIC – Minimum Inhibitory Concentration

OIE – World Organization for Animal Health

PCR – Polymerase Chain Reaction

PCU – Population Correction Unit

SVA – The National Veterinary Institute of Sweden

WHO – World Health Organization

Abbreviations for antibiotic substances

AMP – Ampicillin

AXC – Amoxicillin-clavulanic acid

AZI – Azithromycin

CAZ – Ceftazidime

CHL – Chloramphenicol

CIP – Ciprofloxacin

COL – Colistin

COT – Cefotaxime

COX – Cefoxitin

CTI – Ceftiofur

CTR – Ceftriaxone

FLO – Florfenicol

GEN – Gentamicin

KAN – Kanamycin

NAL – Nalidixic acid

SSZ – Sulfisoxazole

STR – Streptomycin

SUA – Sulfonamides

SUM – Sulfamethoxazole

TET – Tetracycline

TRIM – Trimethoprim

TRSU – Trimethoprim-sulfamethoxazole

INTRODUCTION

Antimicrobial resistance

Antimicrobial resistance (AMR) is a trait among microorganisms which refer to their ability to survive antimicrobial agents that they originally were sensitive to. In bacteria these features are gained naturally through chromosomal mutations or gene transfer from one bacterium to another (Furuya & Lowy, 2006). AMR has raised concerns for decades since patients infected with resistant bacterial strains may not respond well to treatment resulting in impaired recovery or death (EFSA, 2011).

The World Health Organization (WHO) reported in 2013 that antibiotic-resistant infections cost an estimated 1,500 million EUR in the European Union and 2,000 million USD in Thailand each year (WHO, 2013). In the same report WHO states that nosocomial infections with multidrug resistant bacteria cause 30,000 deaths every year in Thailand, more than 25,000 in EU, more than 23,000 in USA and approximately 80,000 in China.

A renowned reason to the development and dissemination of AMR is the widespread use of antimicrobials in livestock (FAO & WHO, 2015). A publication involving seven European countries was issued quite recently showing a high degree of correlation between antibiotic usage and antibiotic resistance in food producing animals (Chantziaras *et al.*, 2014). Van Boeckel *et al.* (2015) were the first to assess the average global consumption of antimicrobials in food animals per year by statistical calculations and estimated that $63,151 \pm 1,560$ tons were used in 2010. In the same report they predicted that this consumption will increase with 67% between 2010 and 2030.

Particular concerns have been raised regarding the use in healthy animals to enhance their growth, productivity and reproduction. In 1969 the Swann Committee became the first to report concerns about the subtherapeutic antibiotic use in livestock and the sequent risks of selecting resistant bacterial strains that possibly could infect humans and result in treatment failure in human medicine (Swann, 1969: see Adjiri-Awere & Van Lunen, 2005). Several antibiotics are used for this purpose on a daily basis in subtherapeutic doses in the animal feed. Due to the risk to increase the AMR growth promoting antibiotics (GPAs) are not allowed in the EU since 2006¹ although some countries banned GPA long before this legislation and Sweden was the first to proscribe GPA in 1986 (Cogliani *et al.*, 2011).

Antibiotics are also given as prophylaxis in low dose to healthy livestock to prevent disease especially in high population densities. However, this doesn't differ from GPA from a microbial perspective (You & Silbergeld, 2014).

On the World Veterinary Day 2012, the Food and Agriculture Organization of the United Nations (FAO) stated that it is now well-established that the imprudent use of antimicrobials may result in AMR (FAO, 2015). Several studies indicate that appearing resistant bacteria in humans may have a food-animal origin and thereby support the theory of dissemination of AMR bacteria from food animals to humans (Ramchandani *et al.*, 2005; Smith *et al.*, 2009; Bezanson

¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition

et al., 1983). FAO has formed a tripartite together with WHO and OIE with a “One Health” approach which aims to coordinate the public health sectors, animal health sectors and the food safety in a global manner. The Tripartite consider the AMR a priority topic for concerted actions and has since 2003 provided information about AMR as well as developed recommendations and guidelines to prudent use of antimicrobials (WHO, 2014).

Nevertheless, there have been debates on whether or not the antibiotic use in animals poses an actual threat to human health. Phillips *et al.* (2004) question this statement and conclude that even though resistance may spread the possibility of harm in human is low. However, they also emphasize the importance of food hygiene, prudent antibiotic use as well as surveillance of diseases and AMR.

Objectives of field work

This is primarily a descriptive study which aims to fill in some of the empty spaces regarding the antibiotic resistance among livestock in Thailand. The antibiotic usage in Thailand and Sweden differs markedly, which is why we also investigated whether the resistance pattern differs accordingly. Additionally the results were compared with data on indicator *E. coli* from Europe and Canada. Those areas were chosen because of their size and location (relatively large areas on two different continents) and also because of their well collected and accessible surveillance data.

Furthermore, the author wanted to identify possible correlations between antibiotic resistance and farm management including antibiotic usage, therefore a questionnaire was included in the study.

Parallel with this study, another study was performed in the same area, which had the same objectives as this study although with backyard small-scale-farms instead of medium-sized farms (Karlsson forthcoming). In backyard farming there are not always fattening pigs and therefore, to reach comparable results with that study, samples were collected from sows instead of fattening pigs although sampling from fattening pigs are recommended in the Decision 2013/652/EU².

Considering the available resources, the following sampling strategy was chosen: rectal swabs collected from three healthy sows on each of the 27 farms, 81 samples in total. This aimed to achieve as comparable results as possible with those received in the surveillance programs of EU and Canada as well as in the study regarding the backyard-farming.

Escherichia coli has three characteristics which make it particularly suitable as indicator bacteria: (i) it is commensal and does not commonly affect human nor animal health, (ii) it is easy to culture and (iii) it has the ability to transfer genes encoding antibiotic features to other bacteria and thereby is still relevant in the antimicrobial resistance problem.

The method used in this study is the same as the broth microdilution method issued by the European Committee on Antimicrobial Susceptibility testing (EUCAST) (2003). The method

² Commission implementing decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria [2013] OJ L303/26

is also in accordance with the European Standard (EN ISO 20776-1:2006) (European Committee for standardization, 2006) which thereby enables the results to be compared with European countries including Sweden.

Multidrug resistance

In 2011 an international expert group jointly decided a terminology to be used for multidrug resistance (MDR) or multiresistance and agreed on defining it as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al.*, 2011). This is also the classification used by The National Veterinary Institute (SVA, 2014).

The antimicrobial categories used in this study were as follows:

Category	Antibiotic substance
Aminoglycosides	Gentamicin, streptomycin
Amphenicols	Chloramphenicol, florfenicol
Carbapenems	Meropenem
Cephalosporins	Cefotaxime, ceftazidime
Folate pathway inhibitors	Trimethoprim, sulfamethoxazole
Penicillins	Ampicillin
Polymyxins	Colistin
Quinolones	Ciprofloxacin, nalidixic acid
Tetracyclines	Tetracycline

LITERATURE STUDY

Pig industry in Thailand

Industrialization of pig farming started in 1973 and the development of large-scale farms accelerated in the 1980s (Cameron, 2000). Nowadays pig production in Thailand is widespread. It should be noted that the estimated numbers from this industry are somewhat scattered depending on source and reporting frequency etc. Therefore one might question the accuracy of them. The collected information is presented below without further assumptions or conclusions.

According to Cameron (2000) 80% of pigs produced in Thailand were from intensive farming systems at the time for its publication. More than half of these were from farms with >1000 pigs (8.5% from company owned farms and 47.5% from privately owned farms). The remaining 44% of pigs from intensive farming systems were from farms with 50-1000 pigs.

Thailand produced more than 16 million fattening pigs in 2013 and the export value of pigs, pork and products was worth 4.5 billion THB (Tantasuparuk & Kunavongkrit, 2014). According to FAO the export value of live animals (pigs) was in 2013 approx. 32 million USD (FAOSTAT, 2016), equivalent to ~26 % of the total export value from the Thai pig and pork industry.

Data from the Thai Department of Livestock Development (DLD) shows a number of 9.5 million pigs in 191,454 households in 2014 (National Institute of Animal Health, 2014). According to Tantasuparuk & Kunavongkrit (2014) 94% of the farms held less than 50 pigs each in 2013, while 0.1% held more than 5000 pigs. The same authors stipulated that the number of large-scaled productions had increased and might continue to do so.

Reproductive performance and diseases in Thailand

Thailand import sows from the same sources as Europe yet the reproductive performance are not as high in Thailand as in Europe and the litter sizes are smaller (Tantasuparuk & Kunavongkrit, 2014) which could be a result of the hot and humid weather (Suriyasomboon *et al.*, 2006). The climate is suitable for bacterial and fungal growth which may affect the reproduction of sows and the quality of feed (Tantasuparuk & Kunavongkrit, 2014). Reproductive performance in sows in Thailand

Table 1. *Reported disease occurrences in small animals in Thailand. The numbers are collected from the Annual report of National Institute of Animal Health (2014). Pigs represented 95.0% of the included animals in the report.*

Disease	Number of diagnosed cases
Rabies	182
PRRS	40
Classical swine fever	17
Porcine circovirus	32
PMWS (Circovirus infection)	1
Porcine parvovirus	1
Colibacillosis	12
Edema disease	15
Streptococcosis	5
Clostridial infection	1
Pasteurellosis	3
Salmonellosis	1
Bacterial infection	2
Glasser's disease	4
Swine dysentery	1
Total	317

is lowered due to several diseases including classical swine fever, foot and mouth disease, PMWS (porcine circovirus), porcine epidemic diarrhea and PRRS (porcine reproductive and respiratory syndrome).

Reported disease occurrences in “small animals” (of which pigs represent 95%) are displayed in a publication issued by the National Institute of Animal Health (2014) and shown in Table 1. Note that this is diagnosed and reported cases, not a complete picture of disease incidence in Thailand.

Surveillance of antibiotic usage in animals and livestock and antimicrobial resistance in Thailand

The Thai Department of Livestock Development (DLD) cooperates with the Thai Food and Drug Administration (TFDA), Ministry of Public Health in the regulation of veterinary drugs (FAO, 2014a). DLD is responsible for the control and surveillance of the usage of veterinary drugs, furthermore they list drugs and chemicals that are not permitted for use in food animals. The TFDA has responsibility for licensing and registration of veterinary medicinal products and authorizes officials of DLD.

No national surveillance and data collection system exists for antimicrobial resistance in livestock and livestock products, although there have been some studies on AMR performed in Thailand, according to the review published by FAO (2014a). Suggested challenges include the lack of standardized and harmonized methods for antimicrobial susceptibility testing, the insufficient regulations and unclear picture of antimicrobial usage in farm animals as well as personnel with inadequate competence.

Nevertheless, there are ongoing undertakings in this area. The government of Thailand has included surveillance of AMR in human and animals in the National Strategic Plan for Emerging Infectious Disease (Bureau of Emerging Infectious Disease, Department of Disease Control. Ministry of Public Health, 2013), the DLD is currently working on a project to harmonize the monitoring of AMR in Thailand and the National Institute of Animal Health (NIAH), established by DLD, is responsible for the surveillance of antibiotic susceptibility (National Institute of Animal Health, 2016). Guidelines of judicious antimicrobial use in poultry have had a positive response among Thai producers and there are ongoing preparations of guidelines customized for other livestock animals. Furthermore, the TFDA has banned the usage of antibiotics for growth promotion purposes in food animals.

The National Antimicrobial Resistance Surveillance Center, Thailand (NARST) receives data on antimicrobial resistance from several hospitals in the country. With support from the WHO the program was initiated in 1998 to investigate the antimicrobial susceptibility of various microorganisms (Dejsirilert *et al.*, 2009). Moreover, Thailand has joined the global antibiotic resistance surveillance program SMART (Study for Monitoring Antimicrobial Resistance Trends) that started in 2002 and was initiated to monitor antimicrobial resistance and epidemiological trends among patients with intraabdominal infections (Hsueh, 2012). However, these programs monitor only human hospitals and thereby only include unhealthy individuals that likely are under treatment.

According to FAO (2014a) the resistance pattern in commensal *E. coli* from livestock is well studied in South, East and Southeast Asia and the results have shown a high resistance in *E. coli* to amoxicillin, ampicillin and tetracycline in all of the studies reviewed. However, only two studies on AMR in *E. coli* from pigs in Thailand were included in that review and the names of those studies were not specified which hinder further inquiry.

Antibiotic usage and surveillance in pigs in Sweden

In 2015 data about the Swedish antibiotic consumption for pigs showed that 2883 kg active antibiotic substance was used in 2014 and 75% (approx. 2160 kg) of these were injectable products (SVA, 2015). Benzylpenicillin was the most commonly used antibiotic substance, 60% of all the injectable antibiotic products sold contained benzylpenicillin. Fluoroquinolones are not commonly used in pigs in Sweden (3.2 kg active substance in 2014) and no usage of third generation cephalosporins in pigs are reported. Over time, the selling of antibiotic products for group medication has decreased. However, the sales of benzylpenicillin for injection has instead increased and so has the total amount of injectable antibiotics, therefore the total antibiotic consumption in pigs has been rather constant over the last years.

Antibiotic administration via feed or water contributes to selection of resistant bacteria in a higher degree compared with individual treatment with narrow-spectrum injectable antibiotics, e.g. benzylpenicillin (SVA, 2015). Thus the change from group medication to individual treatments is beneficial even though the total amount of antibiotics has not changed.

The latest publication from the surveillance of AMR in indicator *E. coli* from pigs in Sweden was issued in 2012 (SVA, 2012). The results from that publication are presented in Table 2 and 3 and further explained under Discussion.

Antimicrobial usage in Sweden is low compared to other countries in the EU. In the fifth ESVAC report regarding sales of veterinary antimicrobial agents (EMA & ESVAC, 2015) Sweden is reported to have the third lowest usage of veterinary antimicrobials among 26 European countries – 12.6 mg/population correction unit (PCU) compared to an average of 109.7 mg/PCU. Population correction unit (PCU) is purely a technical term, used by EMA & ESVAC, to take into account the animal demographics in individual countries. One PCU corresponds to one kg animal weight.

Antibiotic susceptibility in Canada, Europe, Sweden and Thailand

Antibiotic usage differs markedly between Thailand and Sweden, which makes it interesting to investigate whether the resistance pattern differs accordingly. Europe and Canada were chosen for comparison because of their size and location (relatively large areas on two different continents) and also because of their well collected and accessible surveillance data.

The resistance pattern for the different areas is shown in Table 2 and 3.

Canada

In the Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report 2013 (CIPARS, 2015), 1573 isolates of *E. coli* from pig feces were tested for antimicrobial susceptibility. Composite fecal samples were collected from 6 pens with grower-finisher pigs once per year. The antibiotic susceptibility methods were essentially the same as used in this study. Although it needs to be taken into account that the breakpoints applied in that report, were in general a little higher than the ECOFFs presented in this study (especially ciprofloxacin where the breakpoint is considerably higher than the ECOFF), therefore some of the strains that were classified as resistant in this study would not be considered resistant in the Canadian

report. As a result the Canadian numbers in Table 2 and 3 might be lower than it would be with ECOFFs.

Europe

The European report (EFSA & ECDC, 2015) included 1954 isolates of *E. coli* from 10 member states in reporting AMR. Isolates originated from either fattening pigs (seven countries) or breeding animals (one country) or un-specified production type (two countries). The majority of samples were collected randomly from healthy slaughter pig carcasses at the slaughterhouse. Sample collection was relatively evenly distributed over the year. Belgium, Hungary and Poland did not report detailed information on sampling stage, sample type or sampling context. In the analysis of MDR, 1312 isolates from seven countries were included. As in this study, ECOFFs were used for interpretation. In 2013 broth microdilution methods were established as the harmonized method for testing antibiotic susceptibility in EU, although it is not specified whether this method was used or not by the included countries in the report.

Sweden

In the Swedish report (SVA, 2012) 167 isolates of *E. coli* was included. The samples were collected from intestinal content of healthy pigs at slaughter. Each isolate was from a unique herd. Isolation, identification and susceptibility testing were the same as in this study.

Thailand

A Thai study (Jiwakanon *et al.*, 2008) collected 338 isolates between 2003 and 2005 from fecal samples from pig farms in northeastern Thailand. Samples were collected by veterinary service officers and were sent to Veterinary Research and Development center, Upper Northeastern region. Collection method was not mentioned neither were the number of samples per farm. Antimicrobial susceptibility was tested with a disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS), now called Clinical Laboratory Standard Institute (CLSI). The breakpoints used for interpretation were also according to NCCLS.

Table 2. Antimicrobial susceptibility in pigs in Canada, Europe, Sweden and Thailand. GEN=Gentamicin, KAN=Kanamycin, STR=Streptomycin, AMP=Ampicillin, CTR=Ceftiaxone, COX=Cefoxitin, CTI=Ceftiofur, COT=Cefotaxime, CAZ=Ceftazidime, SSZ= Sulfoxazole, TRSU= Trimethoprim-sulfamethoxazole, SUM=Sulfamethoxazole, SUA=Sulfonamides, CHL=Chloramphenicol, FLO=Florfenicol, COL=Colistin, CIP=Ciprofloxacin, NAL=Nalidixic acid, TET=Tetracycline. Dash mark (-) means non-tested. Numbers are collected from CIPARS, 2015; EFSA & ECDC, 2015; SVA, 2012; Jiwakanon et al., 2008 and this report (Thailand, 2015).

Area

(Number of isolates tested)

Percent of isolate resistant by antimicrobial category

	Amino-Glycosides			Penicillins	Cephalosporines					Folate pathway inhibitors					Amphenicols		Polymyxines	Quinolones		Tetracyclines
	GEN	KAN	STR	AMP	CTR	COX	CTI	COT	CAZ	SSZ	TRSU	TRIM	SUM	SUA	CHL	FLO	COL	CIP	NAL	TET
Canada (1573)	1.0	12.5	34.0	31.1	1.3	1.1	1.1	-	-	45.4	13.4	-	-	-	20.3	-	-	-	0.3	75.4
Europe (1954)	1.8		47.8	30.3	-	-	-	1.3	-	-	-	-	-	42.1	14.7	-	-	6.1	3.8	52.8
Sweden (167)	1	1	16	13	-	-	-	<1	-	-	-	11	-	17	4	0	0	2	2	8
Thailand, 2008 (338)	30.8	40.8	66.3	84.5	-	-	-	0.5	-	-	85.2	-	-	-	-	-	3.5	26.8	37.6	97.9
Thailand, 2015 (81)	7.4		76.5	85.2	-	-	-	1.2	3.7	-	-	70.4	84.0	-	58.0	2.4	0	48.1	30.8	86.3

Table 3. Resistance by number of antimicrobials in pigs in Canada, Europe, Sweden and Thailand. In the Canadian report the resistance against 2 and 3 antibiotics were merged and not divided as in the other reports. Therefore “44.7%” refers to resistance against 2 or 3 antibiotics. Dash (-) means non-evaluated. Numbers are collected from CIPARS, 2015; EFSA & ECDC, 2015; SVA, 2012; Jiwakanon et al., 2008 and this report (Thailand, 2015)

Area (Numbers of isolates tested)	Percentage isolates by number of antimicrobial categories in the resistance pattern				
	0	1	2	3	>3
Canada (1573)	16.5	20.5	44.7	18.3	
Europe (1312)	-	-	-	-	39.9
Sweden (167)	72	9	5	3	10
Thailand, 2008 (338)	-	-	-	-	-
Thailand, 2015 (81)	2.5	0	2.5	8.6	87.7

Studies on ESBL and ESBL_{CARBA}

Extended-spectrum beta lactamases (ESBL) are enzymes that have the ability to hydrolyze betalactam antibiotics including 3rd and 4th generation cephalosporins as well as aztreonam (SVA, 2015-12-08; Rawat & Nair, 2010). The betalactam antibiotics are highly important for human and veterinary medicine and therefore information about resistance against these antibiotics is crucial (WHO, 2011; EFSA, 2011). In case of infection with ESBL-producing bacteria, carbapenems are commonly the treatment of choice. Unfortunately the emergence of strains with acquired resistance against carbapenems has also recently increased, not only in human medicine but such strains have also been detected in animals (Fischer *et al.*, 2012; Guerra *et al.*, 2014). Carbapenemases are enzymes produced by the bacteria and are able to hydrolyze carbapenems. Strains that produce carbapenemase are named ESBL_{CARBA}-producing.

In 2014 a study on prevalence of antimicrobial resistance in pigs in Thailand was carried out (Boonyasiri *et al.*, 2014). Rectal swabs were collected from randomly chosen healthy pigs (n=314) in northern and eastern Thailand, one swab per pig. ESBL production was detected by the double disk diffusion method and antibiotic susceptibility was determined with a disk diffusion method. Resistance against cephalosporins was detected by using selective medium agar containing cephalosporins. Important to note is that the high number of isolates tested with such method is likely to entail a higher frequency of resistance against cephalosporins than the method used in this study. Therefore, the respective results are not comparable. Nevertheless, 76.7% of the obtained isolates were found to be ESBL-producing. Two strains were resistant against carbapenem but it is not specified however these strains also were ESBL-producing. The carbapenem resistance was also not confirmed with PCR and therefore it is not possible to tell whether the resistance was caused by a carbapenemase or not.

Fischer *et al.* (2012) and Roschanski *et al.* (in press) report findings of carbapenem resistant strains in pigs from other countries. Lay *et al.*, (2012) and Jiwakanon *et al.* (2008) have studied antimicrobial resistance in pigs in Thailand but resistance against carbapenems was not included in those reports.

MATERIAL AND METHODS

Field sampling

A total of 27 medium-sized farms (100-500 sows) were visited for sample collection between 17th September and 1st October in 2015. Only one farm was private owned whereas the remaining twenty-six were contract farms (i.e. farms owned by companies). The companies are called A and B and owned fourteen and twelve of the contract farms, respectively. From each farm rectal swabs were collected from three healthy sows by veterinary assistants who were thoroughly instructed beforehand how to collect the samples in a correct manner. The samples were then transported to the Khon Kaen University in tubes containing Aimes medium, for analysis. Duration of transport was 1-6 hours. The samples were stored in 2-8°C in maximum 48 hours before analyses.

Isolates

Commensal *Escherichia coli* were used as indicator bacteria. Each fecal sample was streaked on MacConkey agar and incubated at 44°C overnight. At least four colonies with typical morphological appearance consistent with *E.coli* were thereafter sub-cultured on blood agar and incubated at 37°C overnight. To confirm growth of *E.coli*, the isolates were tested for production of tryptophanase through incubation in Motility-Indole-Lysine (MIL) broth at 37°C overnight, followed by addition of Kovac's indole reagent. One confirmed *E. coli* isolate from each sample were further tested for antimicrobial susceptibility.

Antimicrobial susceptibility testing

The medium used was cation-adjusted Mueller-Hinton broth (CAMHB) (Difco) with pH 7.2-7.4. As control strain, *E. coli* CCUG 17620 (ATCC 25922) was included. Bacteria were collected by touching 3-5 colonies on the blood agar with a plastic loop (1 µl) and suspended in 5 ml CAMHB followed by incubation for 1h and 50min at 37°C to reach a concentration of at least 10⁸ CFU/ml. From the preculture 10 µl was transferred to 10 ml CAMHB to obtain a final inoculum density of approximately 5 x 10⁵ CFU/ml. The density was confirmed regularly by taking 10 µl of the inoculum and diluting it in 10 ml 0.9% saline. From this dilution 100 µl was spread on a blood agar plate and an interval of 10-100 CFU was considered as an acceptable inoculum variation.

Broth microdilution was performed with VetMIC GN-mo panels, manufactured by The National Veterinary Institute, Uppsala, Sweden. Each VetMIC-plate consists of 12x8 wells with different antibiotic substances in serial twofold dilutions in each column (Appendix 1) with raising concentration of active substance in the direction H-A.

Each well was filled with 50 µl of the inoculum. The wells were sealed with a transparent covering tape and the panels were incubated for 17 hours at 36°C. After 17 hours the bacterial growth in the wells was investigated and the lowest antibiotic concentration that totally inhibited visible growth of bacteria was noted as the minimum inhibitory concentration (MIC).

Cut-off values

Cut-off values are used in the interpretation of resistance in bacteria. If the MIC exceeds the cut-off value the bacterial strain classifies as resistant, if not it classifies as sensitive. The cut-off values in this report are the epidemiological cut-off values (ECOFFs) published by EUCAST on their webpage (www.eucast.org), presented in Table 4 below.

Table 4. *Epidemiological cut-off values for the included antimicrobials* (www.eucast.org)

Antimicrobial agent	ECOFFs
Ampicillin	>8
Ciprofloxacin	>0.06
Nalidixic acid	>16
Gentamicin	>2
Streptomycin	>16
Tetracycline	>8
Florfenicol	>16
Colistin	>2
Sulfamethoxazole	>64
Trimethoprim	>2
Chloramphenicol	>16
Meropenem	>0.12
Cefotaxime	>0.25
Ceftazidime	>0.5

In this report the word resistant is used for isolates with reduced susceptibility for an antibiotic substance. Important to note to avoid misunderstanding is that resistance in this context does not always suggest clinical resistance. ECOFFs are set to find all the isolates that may have a resistance mechanism.

Questionnaire

The owner was interviewed regarding the farm structure, pig husbandry and the routines for antibiotic treatment (Appendix 2). The questions were asked by authors to this article together with a Thai speaking veterinary student who translated the questions to Thai and the farmer's answers to English.

To make sure the questions were well understood and easy to answer the questionnaire was first piloted on another farm, not connected to the study.

Statistical analysis

Statistical analysis was conducted in SAS software 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics were computed to define farm characteristics. To investigate associations between management factors, such as use of antibiotics and veterinary services, with resistance against different types of antibiotics, 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 management and veterinary service

A-farms

The A-farms were breeding farms (i.e. produce weaned pigs that are sold to fattening farms). Eight of 14 farms held 100-200 sows, four held 201-300 sows and two held 301-400 sows. The sows were obtained from the company. It was only possible to get information from one A-farm about the weaned pigs – that farm had 300 sows and produced 600-700 weaned pigs each month. The sows were vaccinated against foot-and-mouth disease (FMD), atrophic rhinitis (AR), classical swine fever (CSF) and Aujeszky's disease (AD). Hygiene and cleaning routines were based on a continuous flow system. Feces were removed from the floors every day. The floors of the gestation sows were cleaned with water every second or third day and the floors of the nursing sows were disinfected with glutaraldehyde to prepare farrowing unit. Evaporation system was used as cooling system. The nursing sows had concrete slatted floor and were confined in farrowing crates. The gestation sows had solid concrete floor and were confined in gestation crates.

B-farms

The B-farms were breeding farms (i.e. produce weaned pigs that are sold to fattening farms). Three of the twelve B-farms held 100-200 sows and nine held 201-300 sows. Five farms produced 400-600 weaned pigs each month from 201-300 sows, no information was obtained from the other seven farms. The sows were obtained from the company. All of the B-farms vaccinated their sows against FMD, CSF and AD. Hygiene and cleaning routines were based on a continuous flow system. Feces were removed from the floors every day. The gestation barns were cleaned twice a week with water, detergent and glutaraldehyde and the same protocol were used for the nursing pens after weaning, following 7 days where the pen was left empty before next sow. They used evaporation system to cool the air. The nursing sows had concrete slatted floor and were confined in farrowing crates. The gestation sows had solid concrete floor and were confined in gestation crates.

Private owned farm

This farm held approx. 400 sows which were bred on the farm and produced 800-1000 weaned pigs each month. The farm was a combined breeding and farrow-to-finish farm. They vaccinated the sows against FMD, CSF, Pseudorabies, Porcine Reproductive and Respiratory Syndrome (PRRS), Porcine Circovirus (PCV) and Porcine Parvovirus (PPV). Hygiene and cleaning routines were based on a continuous flow system. Feces were removed from the floors every day and they also cleaned with water every day. Once a week the floors were disinfected with glutaraldehyde. Water and disinfection were used after weaning and the pen was left empty for 7 days before next sow. For cooling, the private owned farm used conventional (open air) system with fans. The nursing sows had slatted floor and were confined in farrowing crates. The gestation sows had slatted floor at the back of their pens, but solid concrete at the front and were confined in gestation crates.

Location

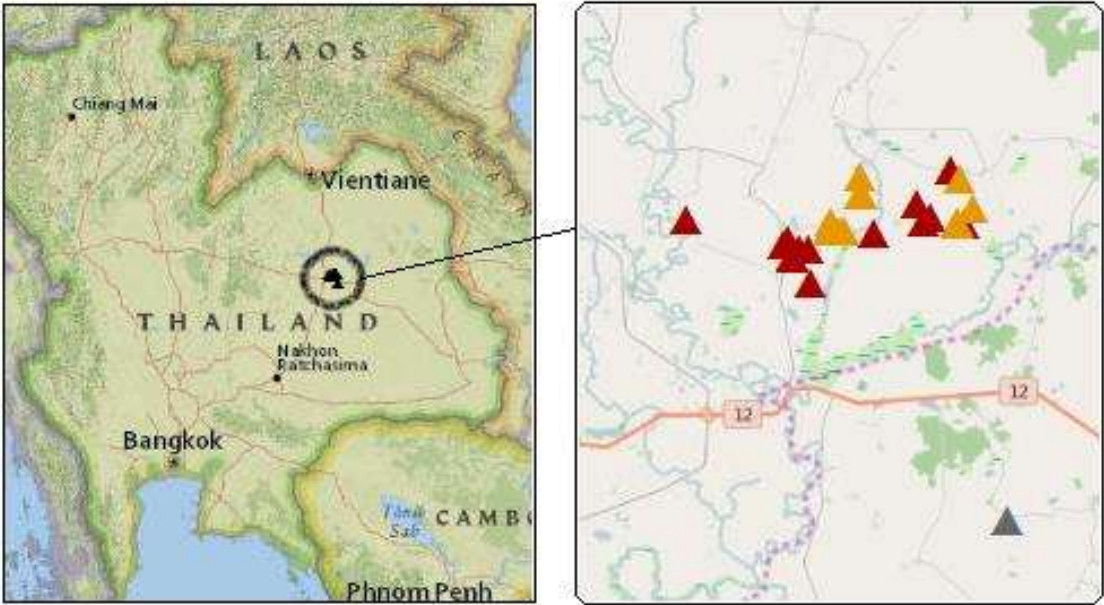


Figure 1. Overview of the locations of the farms. Red: A, orange: B, grey: private owned.

Antibiotic usage

Among the farms in this study, penicillin and streptomycin were the most commonly used antibiotic substances for injection. The two companies had their own separate regimes regarding the antibiotic use but they had the same regimes on all of their farms respectively.

A-farms

A-farms (14 farms) administered Kitamycin in feed for both nursing and gestation sows (Figure 3). Penicillin and streptomycin were administered as injection for preventive purpose after farrowing (Figure 2). They obtained antibiotics from the company and the veterinarian at the company decided when and how to give antibiotics.

B-farms

B-farms (12 farms) used penicillin, streptomycin and enrofloxacin for injection when sows were ill (Figure 2) and oxytetracycline in the daily feed for nursing sows (Figure 3), but no antibiotics in the feed for gestation sows. No answers were obtained from the B-farms whether they gave antibiotics to sows as preventive treatment after farrowing. The antibiotics were obtained from the company and the veterinarian at the company decided when and how to give antibiotics.

Private owned farm

The private owned farm used penicillin and streptomycin for injection as preventive treatment after farrowing and amoxicillin, oxytetracycline and cefotaxime for injection as treatment when sows were ill (Figure 2). In the daily feed for both nursing and gestation sows, they

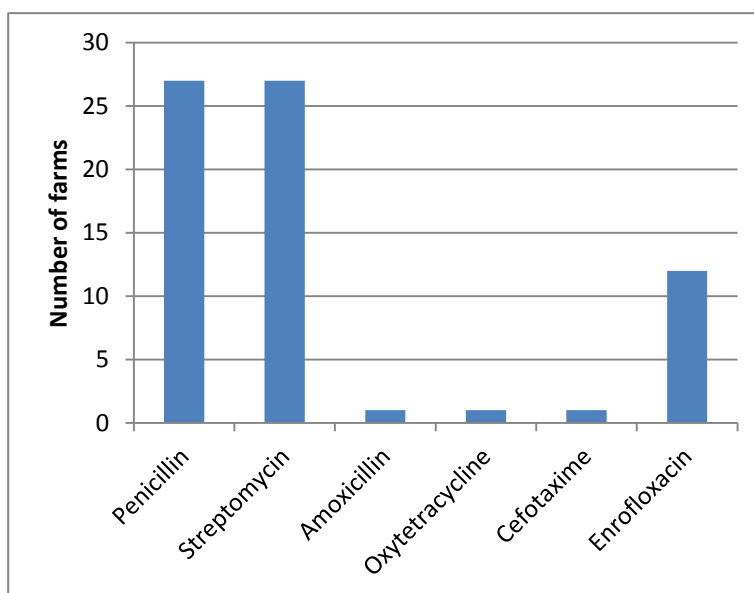


Figure 2. Number of farms administrating antibiotics as injection in preventive purpose and for treatment. The columns represent number of farms using a specific antibiotic. Penicillin and streptomycin were used at 27 farms, enrofloxacin was used at twelve farms and amoxicillin, oxytetracycline and cefotaxime were used at one farm.

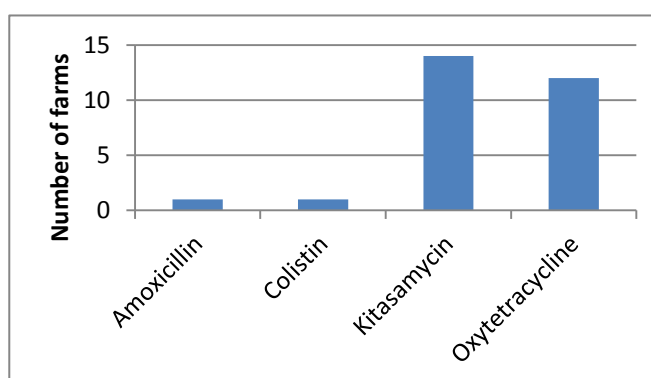


Figure 3. Number of farms administrating antibiotics in the daily feed for nursing sows. Amoxicillin and colistin were given at one farm whereas kitasamycin and oxytetracycline were given at 14 and 12 farms respectively.

administered amoxicillin and colistin (Figure 3). This farm was owned by a veterinarian who also was responsible for the antibiotic regimes and treatments.

Antibiotic resistance

Escherichia coli was isolated from all 81 samples cultured (100%). Two of the 81 isolates (2.5%) were susceptible to all antibiotics and 77 (95.1%) of the samples were multidrug resistant (MDR) i.e. resistant to three or more antibiotic categories (Table 5). The antibiotic substances that the isolates most commonly were resistant to were tetracycline and ampicillin – 86.3% and 85.2% respectively (Table 7).

Distributions of MICs for different antibiotics are presented in Table 7.

Results of antibiotic susceptibility on each farm are described below and presented in Table 8.

One of the isolates (M13) was resistant to meropenem (MIC >0.25) as well as to the other β -lactam antibiotics (ampicillin, ceftazidime and cefotaxime). The MICs of the tested antibiotics for this strain are shown in Table 6.

Table 5. Multidrug resistance (MDR) in *Escherichia coli*. The table shows the number of isolates and the proportion (%) of isolates resistant to none or several of the antibiotic categories tested.

Number of antibiotic categories to which an isolate was resistant	Number of isolates	Percent
0	2	2.5
1	0	0
2	2	2.5
≥ 3	77	95.1

Table 6. MIC of the tested antibiotics for *Escherichia coli* strain M13. The results show a high MIC (>0.25) of meropenem.

Antibiotic substance	MIC
Ampicillin	>128
Ciprofloxacin	1
Nalidixic acid	128
Gentamicin	1
Streptomycin	128
Tetracycline	32
Florfenicol	8
Colistin	2
Sulfamethoxazole	>1024
Trimethoprim	>16
Chloramphenicol	16
Meropenem	>0.25
Cefotaxime	>2
Ceftazidime	>4

Table 7. Resistance and distributions of minimum inhibitory concentrations (MICs) for the isolates tested (n=81). White fields denote range of dilutions tested for each substance. MICs higher than the highest concentration tested are given as the concentration closest above the range. MICs equal to or lower than (\leq) the lowest concentration tested, are given as the lowest tested concentration (underlined). The ECOFF for each substance is presented as a vertical line.

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	85.2							<u>4.9</u>	8.6	1.2					2.5	82.7				
Ciprofloxacin	48.1			9.9	42	4.9	4.9	23.5	6.2	8.6										
Nalidixic acid	30.8									14.8	35.8	14.8	3.7	1.2	1.2	4.9	23.5			
Gentamicin	7.4						<u>1.2</u>	29.6	51.9	9.9	3.7	1.2		2.5						
Streptomycin	76.5										3.7	4.9	14.8	27.2	14.8	13.6	8.6	12.3		
Tetracycline	86.3								<u>2.5</u>	3.7	3.7	3.7	12.3	22.2	37	14.8				
Florfenicol	2.4										<u>7.4</u>	38.3	51.9	1.2	1.2					
Colistin	0						<u>75.3</u>	19.8	4.9											
Sulfamethoxazole	84											<u>6.2</u>	8.6	1.2						84.0
Trimethoprim	70.4					6.2	18.5	4.9						70.4						
Chloramphenicol	58										<u>9.9</u>	21.0	11.1	45.7	12.3					
Cefotaxime	1.2		<u>7.4</u>	56.8	33.3	1.2					1.2									
Ceftazidime	3.7			<u>1.2</u>	1.2	66.7	27.2	2.5				1.2								

A-farms

Highest resistance rate seemed to be for sulfamethoxazole to which 39 of 42 isolates (92.9%) were resistant. The three isolates that were susceptible to sulfamethoxazole were also susceptible to the other folate pathway inhibitor, trimethoprim. The isolates had also generally high resistance rate to ampicillin; 35 of 42 isolates (83.3%) were resistant. To tetracycline 32 of 42 isolates (76.2%) were resistant. Streptomycin resistance appeared in 28 of 42 isolates (66.7%), whereas the other aminoglycoside gentamicin had a generally low MIC and all isolates were sensitive. Resistance to chloramphenicol was found in 23 of 42 (54.8%) isolates but only one isolate were also resistant to the other amphenicol, florfenicol. Of the 42 isolates 15 (35.7%) were resistant to at least one of the quinolones, ciprofloxacin and nalidixic acid. Meropenem resistance was found in one single isolate (isolate M13, presented in Table 6), this isolate was also resistant to the other betalactams (ampicillin, cefotaxime and ceftazidime). No resistance was found against colistin.

B-farms

All of the 36 isolates (100%) were resistant to tetracycline. Many isolates were also resistant against streptomycin; 31 of 36 isolates (86.1%). Five were resistant to gentamicin and all of them were resistant to streptomycin as well. A generally high resistance rate was also seen against ampicillin (31 of 36, 86.1%). Sulfamethoxazole and trimethoprim (folate pathway inhibitors) resistance appeared in 26 of 36 isolates (72.2%) respectively and 31 isolates (86.1%) in total. Of the isolates 23 (63.9%) were resistant to ciprofloxacin and 14 of them (38.9%) were also resistant to the other quinolone, nalidixic acid. Chloramphenicol resistance appeared in 21 of 36 isolates (58.3%) however no isolate was resistant to florfenicol. No isolate was resistant to cefotaxime. Ceftazidime resistance appeared in one single isolate (2.8%), which was also resistant to ampicillin. No isolate was resistant to colistin.

Private owned farm

All of the three isolates (100%) were resistant to ampicillin, streptomycin, sulfamethoxazole, trimethoprim and chloramphenicol. Two of these isolates (66.7%) were also tetracycline resistant and one of them (33.3%) was furthermore resistant to the quinolones, gentamicin and florfenicol. None of the isolates were resistant to neither colistin nor the cephalosporins.

Table 8. Number of resistant and susceptible isolates found on each farm. Three isolates from each farm were tested for susceptibility against the antibiotic substances below. R=resistant isolate, S=Susceptible isolate. Amp=Ampicillin, Cip=Ciprofloxacin, Nal=Nalidixic acid, Gen=Gentamicin, Str=Streptomycin, Tet=Tetracycline, Flo=Florfenicol, Col=Colistin, Sum=Sulfamethoxazole, Trim=Trimethoprim, Chl=Chloramphenicol, Cot=Cefotaxime, Caz=Ceftazidime.

Farm ID	Amp	Cip	Nal	Gen	Str	Tet	Flo	Col	Sum	Trim	Chl	Cot	Caz
A 1	RRR	RSS	SSS	SSS	SSS	RRR	SSS	SSS	RRR	RSS	SSS	SSS	SSS
A 2	SSS	RSS	SSS	SSS	RSS	RSS	SSS	SSS	RSS	RSS	RSS	SSS	SSS
A 3	RRR	SSS	SSS	SSS	RRS	RRR	SSS	SSS	RRS	RRS	RRS	SSS	SSS
A 4	RRS	SSS	SSS	SSS	RRS	RRS	SSS	SSS	RRR	RRR	RRS	SSS	SSS
A 5	RRR	RSS	RSS	SSS	RSS	RRR	SSS	SSS	RRR	RRR	RRS	RSS	RSS
A 6	RRR	RRS	RSS	SSS	RRS	RRS	SSS	SSS	RRR	RRS	RRR	SSS	SSS
A 7	RRR	RRR	RRR	SSS	RRR	RRS	SSS	SSS	RRR	RSS	SSS	SSS	SSS
A 8	RRR	RRS	RRS	SSS	RRR	RRS	SSS	SSS	RRR	RSS	RRS	SSS	SSS
A 9	RRS	SSS	SSS	SSS	RSS	RRS	SSS	SSS	RRR	RRS	RRS	SSS	SSS
A 10	RRS	RSS	RSS	SSS	RRR	RRR	SSS	SSS	RRR	RRS	RRS	SSS	SSS
A 11	RRR	SSS	SSS	SSS	RRR	RRS	SSS	SSS	RRR	RSS	RSS	SSS	SSS
A 12	RRR	RSS	RSS	SSS	RRR	RRS	RSS	SSS	RRR	RRR	RRR	SSS	SSS
A 13	RRR	RRS	RSS	SSS	RRS	RRR	SSS	SSS	RRR	RRR	RSS	SSS	SSS
A 14	RRS	RSS	SSS	SSS	RRS	RRS	SSS	SSS	RRR	RRR	RRS	SSS	RSS
B 1	RRS	RSS	SSS	RSS	RRR	RRR	SSS	SSS	RRS	RSS	RRS	SSS	SSS
B 2	RRR	RSS	RSS	RRS	RRR	RRR	SSS	SSS	RRR	RRS	RRS	SSS	SSS
B 3	RRR	RRS	RSS	SSS	RSS	RRR	SSS	SSS	RSS	RRS	RRS	SSS	SSS
B 4	RRR	RRR	RSS	SSS	RRR	RRR	SSS	SSS	RSS	RSS	SSS	SSS	SSS
B 5	RRR	RSS	SSS	SSS	RRR	RRR	SSS	SSS	RRS	RRR	RRS	SSS	SSS
B 6	RRR	RRR	RSS	RSS	RRR	RRR	SSS	SSS	RRS	RRS	RRS	SSS	SSS
B 7	RRS	RRS	RRS	SSS	RRR	RRR	SSS	SSS	RRR	RRS	RRS	SSS	RSS
B 8	RRR	RRS	RSS	SSS	RRR	RRR	SSS	SSS	RRR	RRR	RRS	SSS	SSS
B 9	RRS	RRR	RRS	SSS	RRR	RRR	SSS	SSS	RRR	RRR	RRS	SSS	SSS
B 10	RSS	RRS	RRS	SSS	RSS	RRR	SSS	SSS	RRS	RSS	RSS	SSS	SSS
B 11	RRR	RRS	RRS	RSS	RRR	RRR	SSS	SSS	RRR	RRR	RRR	SSS	SSS
B 12	RRR	RSS	RSS	SSS	RRS	RRR	SSS	SSS	RSS	RRR	RSS	SSS	SSS
Private owned	RRR	RSS	RSS	RSS	RRR	RRS	RSS	SSS	RRR	RRR	RRR	SSS	SSS

Relationships between category of farm and resistance patterns

E. coli isolated from the B-category farms were significantly less often resistance to gentamicin compared with the other farm categories (Table 9). No significant relationships between farm category and resistance were found for ampicillin, ciprofloxacin, nalidixic acid, florfenicol, chloramphenicol, tetracycline or ceftazidime.

Table 9. Correlation between farm category and Gentamicin (GEN) resistance. Fisher's exact test is used since there are less than 5 observations in at least one of the boxes. P -value = 0.0061.

Farm category	GEN	
	Susceptible	Resistant
A	8	4
B	14	0
Private	0	1

DISCUSSION

Associations between antibiotic resistance, antibiotic usage and management factors

It should be noted that this is only speculations from observing the obtained results, as the number of farms included in the study were too few and the variations in management and antibiotic usage were too small to obtain statistically significant data. Nevertheless it is interesting to discuss indications of differences between the contract farms and associations between resistance pattern, antibiotic usage and farm management.

In general the resistance pattern is quite similar when comparing the contract farms. B-farms had oxytetracycline in their feed which possibly could explain the high resistance rate to tetracycline (100%) compared to the A-farms (76%). B-farms used, unlike A-farms, enrofloxacin for injection. Enrofloxacin is a quinolone like ciprofloxacin and nalidixic acid. The resistance rate for quinolones in this study was higher in the B-farms (64%) than in the A-farms (36%) which suggests a possible selection of quinolone resistant bacteria due to the usage of enrofloxacin. Furthermore B-farms had a generally high resistance rate against the aminoglycosides (88% of the isolates) compared to A-farms (67% of the isolates). These differences might be due to management factors but since no statistical significance could be obtained from this data no real conclusions can be made about potential associations.

Statistical analysis

The results from these tests are difficult to interpret since the private owned farm is only one farm. However the results indicate that the B-farms were less probable to show resistance against gentamicin, compared to A and the private owned farm.

ESBL and ESBL_{CARBA}

Some of the results for meropenem were found to be unreliable, the meropenem MICs were therefore withdrawn from the study. Nevertheless, one of the isolates (M13) was found to have a high MIC for the betalactam antibiotics including meropenem. Whether this reduced susceptibility is caused by a carbapenemase or not needs to be confirmed through PCR (polymerase chain reaction). In case of confirming an ESBL_{CARBA} it would be interesting to note that none of the farms included in this study used carbapenems for their pigs.

If the strain is found to produce carbapenemase, this study might be the first to report an ESBL_{CARBA}-producing *E. coli* from pigs in Thailand.

Comparison with data from Sweden, Europe and North America

Antimicrobial usage

The prudent use of antimicrobials in Sweden is in contrast to the seemingly widespread use in Thailand. Despite major efforts, no gathered information on antibiotic usage or sales in Thailand has been found, which hinder comparison with Sweden, Europe or Canada. Nevertheless, the results of the questionnaires in this study indicate an extensive use of antimicrobials in pigs, for therapeutic purpose as well as preventive and growth promoting purpose. It is furthermore clear that all of the farms routinely administered antibiotic as injection for preventive purpose after farrowing. Reasons to this wide use could be inadequate information and education among livestock personnel and insufficient national regulation and monitoring. Furthermore, the climate in Thailand promotes bacterial and fungal diseases which might result in increased antimicrobial use.

Antimicrobial susceptibility

As mentioned in the literature study, there are some differences in methods and interpretation between the reports and studies from different countries/areas, although the differences in resistance levels are striking. Sweden has the lowest resistance frequency for all included antibiotics and the lowest frequency of MDR strains (Table 3). In contrast, both studies from Thailand show very high resistance for all antibiotics compared to the other parts of the world. The Thai studies have quite similar levels of resistance where comparison is possible, with the exception for gentamicin for which the study from 2008 shows a substantially higher resistance.

Earlier reports that investigated a possible correlation between antimicrobial usage and AMR indicate that the antimicrobial use correlates with the resistance pattern nationally (Chantziaras *et al.*, 2014). Since Swedish pig industry is prudent in its use of antimicrobials and also has a comparatively low resistance pattern, it is not farfetched to suspect that the extent of resistance in this study and Jiwakanon *et al.* (2008) is influenced by the extensive use of antibiotics in Thailand.

Note that the countries chosen for comparison in this study are selected partly from their data accessibility and do not represent the rest of the world. Such comparison would have to include continents with data limitations and shortage in surveillance systems, e.g. Africa or Asia. Thailand should therefore not be seen as an exception in this matter. Their high levels of resistance possibly match the global situation to a larger extent than Sweden, Europe or Canada.

CONCLUSIONS

Even though there are undertakings regarding the AMR in Thailand, such as the ban of GPAs, the picture of antimicrobial use in animals remains less defined. In the European Union a surveillance program already exists (European Surveillance of Veterinary Antimicrobial Consumption, ESVAC) to identify risk factors for antimicrobial resistance. This surveillance also allows the comparison of antibiotic usage between countries. A similar program would be needed in Thailand as well as other Southeast Asian countries to be able to draw plausible conclusions regarding the effect of antibiotic usage in this region.

This study shows a wide use of antibiotics in the farms included. Not to say the least for preventive purpose after farrowing, where all of the farms administered antibiotics as injection as a routine. The results from the antibiotic susceptibility tests display a generally high MIC, and a high resistance frequency, for a majority of the included antibiotics. These results indicate that a wide use of antibiotics results in resistant bacteria. Therefore, implementing a prudent antibiotic use, as well as surveillance systems, in Thailand, as well as globally is important to curb the development of more resistant bacteria.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Jatesada Jiwakanon, Prof. Ulf Magnusson, Märith Pringle, Lise-Lotte Fernström and Gunilla Ström for all help and support during the preparations and writing of this essay as well as all the respondents to the survey.

REFERENCES

- A.-P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber & D. L. Monnet. (2011). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance.
- Adjiri-Awere, A. & Van Lunen, T. A. (2005). Subtherapeutic use of antibiotics in pork production: Risks and alternatives. *Canadian Journal of Animal Science*, 85:117-130.
- Bezanson, G.S., Khakhria, R. & Bollegraaf, E. (1983). Nosocomial outbreak caused by antibiotic-resistant strain of *Salmonella typhimurium* acquired from dairy cattle. *Canadian Medical Association Journal* 128:426-7.
- Boonyasiri, A., Tangkoskul, T., Seenama, C, Saiyarin, J., Tiengrim, S. & Thamlikitkul, V. (2014). Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathogens and Global Health*, 108(5):235-245.
- Bureau of Emerging Infectious Disease, Department of Disease Control. Ministry of Public Health. (2013). *Thailand's National Strategic Plan for Emerging Infectious Disease. Preparedness, Prevention and Response (2013-2016)*.
- Cameron, R.D.A. (2000). A review of the industrialization of pig production worldwide with particular reference to the Asian region. Focus is on clarifying the animal and human health risks and reviewing the Area Wide Integration concept of specialized crop and livestock activities. FAO:
<http://www.fao.org/ag/againfo/themes/documents/pigs/A%20review%20of%20the%20industrialisation%20of%20pig%20production%20worldwide%20with%20particular%20reference%20to%20the%20Asian%20region.pdf>
- Chantziaras, I., Boyen, F., Callens, B. & Dewulf, J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. *Journal of Antimicrobial Chemotherapy*, 69:827–834.
- CIPARS (Canadian Integrated Program for Antimicrobial Resistance Surveillance), Government of Canada. (2015). 2013 Annual Report – Chapter 2. Antimicrobial Resistance. Public Health Agency of Canada, Guelph, Ontario
- Cogliani, C., Goossens, H. & Greko, C. (2011). Restricting Antimicrobial Use in Food Animals: Lessons from Europe. *Microbe*, 6:274-279.
- Dejsirilert, S., Suankratay, C., Trakulsomboon, S., Thongmali, O., Sawanpanyalert, P., Aswapokee, N. & Tantisiriwat, W. (2009). National Antimicrobial Resistance Surveillance, Thailand (NARST) Data among Clinical Isolates of *Pseudomonas aeruginosa* in Thailand from 2000 to 2005. *Journal of the Medical Association of Thailand*, 92(4):S68-75.

EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control). (2015). EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. *EFSA Journal* 2015;13(2):4036.

EMA (European Medicines Agency) & ESVAC (European Surveillance of Veterinary Antimicrobial Consumption). (2015). Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013. (EMA/387934/2015).

European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (2003). *Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution*.

European committee for standardization. (2006). SS-EN ISO 20776-1:2006. *Clinical laboratory testing and in vitro diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility devices – Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases* (ISO 20776-1:2006).

European Food Safety Authority (EFSA). (2011). *Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β -lactamases and/or AmpC β -lactamases in food and food-producing animals*.

FAO (Food and Agriculture Organization of the United Nations) & WHO (World Health Organization). (2015). *Codex texts on foodborne antimicrobial resistance*.

FAO (Food and Agriculture Organization of the United Nations) (2015-01-07). *FAO statement on World Veterinary Day 2012 – Antimicrobial resistance*.
http://www.fao.org/ag/againfo/programmes/en/empres/news_270412.html [2015-12-18]

FAO (Food and Agriculture Organization of the United Nations). (2014a). *REVIEW of the Literature on Antimicrobial Resistance in Zoonotic Bacteria from Livestock in East, South and Southeast Asia*.

FAO (Food and Agriculture Organization of the United Nations). (2014b). *REPORT on Antimicrobial Usage and Alternatives for Prophylaxis and Performance Enhancement in Pig Populations in East and Southeast Asia*.

FAOSTAT (2016-01-04). *Filters/Trade/Live Animals*.
<http://faostat3.fao.org/download/T/TA/E> [2016-01-04]

Fischer, J., Rodríguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R. & Guerra, B. (2012). Escherichia coli producing VIM-1 carbapenemase isolated on a pig farm. *The Journal of Antimicrobial Chemotherapy*, 67(7):1793-1795.

Furuya, E. Y. & Lowy, F. D. (2006). Antimicrobial-resistant bacteria in the community setting. *Nature Reviews Microbiology*, 4:36-45.

- Grundmann, H., Livermore, D.M., Giske, C.G., Canton, R., Rossolini, G.M., Campos, J., Vatopoulos, A., Gniadkowski, M., Toth, A., Pfeifer, Y., Jarlier, V., Carmeli, Y., the CNSE Working Group. (2010). Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. *Euro Surveillance*, 15(46):pii=19711. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19711>
- Guerra, B., Fischer, J. & Helmuth, R. (2014). An emerging public health problem: Acquired carbapenemase-producing microorganisms are present in food-producing animals, their environment, companion animals and wild birds. *Veterinary Microbiology*, 171:290–297
- Hsueh, P-R. (2012): Study for Monitoring Antimicrobial Resistance Trends (SMART) in the Asia-Pacific region, 2002–2010. *International Journal of Antimicrobial agents*, 40:S1-S3.
- Jiwakanon, N., Limapirak, S. & Soysuwan, C. (2008). Surveillance of antimicrobial resistance in *Escherichia coli* from chicken and pig feces in the upper northeastern region between 2003 and 2005. *Thai-NIAH eJournal*, 2(3):118-129.
- Karlsson, D. (forthcoming). Prevalence of antimicrobial resistance in backyard swine farms in Thailand using *Escheichia coli* as indicator bacteria – a minor field study in antimicrobial resistance. *Swedish University of Agricultural Science, Uppsala, Sweden*.
- Lay, K. K., Koowattananukul, C., Chansong, N. & Chuanchuen, R. (2012). Antimicrobial Resistance, Virulence, and Phylogenetic Characteristics of *Escherichia coli* Isolates from Clinically Healthy Swine. *Foodborne Pathogens and Disease*, 9(11):992-1001.
- Miriagou, V., Cornaglia, G., Edelstein, M., Galani, I., Giske, C. G., Gniadkowski, M., Malamou-Lada, E., Martinez-Martinez, L., Navarro, F., Nordmann, P., Peixe, L., Pournaras, S., Rossolini, G. M., Tsakris, A., Vatopoulos, A. & Cantón, R. (2010). Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. *Clinical Microbiology and Infection*, 16(2):112–122.
- National Antimicrobial Resistance Surveillance Center, Thailand (NARST). (2015-12-14). *Antibiograms*. <http://narst.dmhc.moph.go.th/antibiograms.html> [2015-12-23]
- National Institute of Animal Health (2014). *Annual report, 2014*. Available at: <http://niah.dld.go.th/th/files/stat/pdf/statDiag57.pdf>
- National Institute of Animal Health (NIAH). (2016-01-08). *Organization*. http://niah.dld.go.th/en/index.php?option=com_content&view=article&id=7&Itemid=109 [2016-01-08]
- Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R. & Waddell, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy* 53:28-52.

- Ramchandani, M., Manges, A.R., DebRoy, C., Smith, S.P., Johnson, J.R. & Riley, L.W. (2005). Possible animal origin of human-associated multidrug-resistant, uropathogenic *Escherichia coli*. *Clinical Infectious Diseases*. 40:251-7.
- Rawat, D. & Nair, D. (2010). Extended-spectrum β -lactamases in Gram Negative Bacteria. *Journal of Global Infectious Diseases*, 2(3):263-274. doi:10.4103/0974-777X.68531.
- Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (Text with EEA relevance). (2003) OJ L268/29.
- Roschanski, N., Friese, A., Von Salviati- Claudius, C, Hering, J., Kaesbohrer, A., Kreienbrock, L. & Roesler, U. (in press). Prevalence of carbapenemase producing Enterobacteriaceae isolated from German pig-fattening farms during the years 2011–2013. *Veterinary Microbiology*.
- Smith, T.C., Male, M.J., Harper, A.L., Kroeger, J.S., Tinkler, G.P., Moritz, E.D., Capuano, A.W., Herwaldt, L.A. & Diekema, D.J. (2009). Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strain ST398 Is Present in Midwestern U.S. Swine and Swine Workers. *PLoS ONE*, 4:e4258.
- Suriyasomboon, A., Lundeheim, N., Kunavongkrit, A. & Einarsson, S. (2006). Effect of temperature and humidity on reproductive performance of crossbred sows in Thailand. *Theriogenology*, 65(3):606–628.
- SVA (The National Veterinary Institute) (2015-12-08). *ESBL*. <http://www.sva.se/antibiotika/anmalningspliktig-resistens/esbl> [2015-12-18]
- SVA (The National Veterinary Institute). (2012). *SWARM 2011, Swedish Veterinary Antimicrobial Resistance Monitoring*.
- SVA (The National Veterinary Institute). (2015). *SWEDRES-SVARM 2014. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden. Solna/Uppsala*. ISSN 1650-6332.
- Swann, M. M. (1969). *Joint committee on the use of antibiotics in animal husbandry and veterinary medicine*. Her Majesty Stationary Office, London, UK.
- Tantasuparuk, W. & Kunavongkrit, A. (2014). Pig production in Thailand. *Country Report*. <http://www.angrin.tlri.gov.tw/English/2014Swine/p136-144.pdf>
- Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A. & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America*, 112(18):5649-5654.
- World Health Organization (WHO). (2011). *Critically important antimicrobials for human medicine*. 3rd revision.

World Health Organization (WHO). (2013). Antimicrobial drug resistance Report by the Secretariat. *EB134/37*.

World Health Organization (WHO). (2014). *Antimicrobial resistance: global report on surveillance*. ISBN: 978 92 4 156474 8.

http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1 [2015-12-12].

You, Y. & Silbergeld, E. K. (2014). Learning from agriculture: Understanding low-dose antimicrobials as drivers of resistome expansion. *Frontiers In Microbiology*, 5(284):1-10.

APPENDICES

Appendix 1

Art.no: 395103

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

Note: No growth in the tri-cit control well implies that the strain is sensitive to the citric acid included in the buffer used for Am. In such case, reading of MIC for Am is not relevant. Repeat testing with 100µl per well to dilute the citric acid.

Note! The concentration in the wells will be half of that noted above. Reading of MICs will have to be adjusted accordingly.

Isolate

Date

Remarks

Panel

Mueller Hinton batch

Signature

Appendix 2

Questionnaire – medium-sized farms

Name of the owner of the farm:	GPS-coordinations:
Working position of person answering the questionnaire:	Type of farm (farrow-to-finish/ <u>breeding</u>):
Number of sows:	Number of weaned pigs/month:
1. From where do you get your sows?	
a. Breed our own	
b. From the company	
c. From another farm in the district	
d. From another farm, not in the same district	
e. Other:	

Questions about antibiotic usage

2. Do you give antibiotics in the feed?
Yes/No
3. Which sows do you give antibiotics in the feed?
a. Nursing sows
b. Gestation sows
c. Nursing and gestation
4. 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):

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

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

Per os :

Intramuscular :

6. How do you get access to the antibiotics?

- a. From the company
- b. Buy from veterinarian
- c. Buy from local store/pharmacy
- d. Other:

7. 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	300-400 ppm
Gestation	Times/d	Kg/d	

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

- a. Veterinarian
- b. Small doctor
- c. Foreman
- d. Worker
- e. Other person:

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

Yes/No

Questions regarding husbandry

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

11. How often are the floors
a. cleaned from faeces with a broom (or something similar)?
b. washed with water/soap/disinfection?

- a.
- b.

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

For us to fill in

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

- a. Evaporation

b. Conventional (open air)

c. Other:

14. What type of floor do the sows have?

15. Are the sows confined? What type of confinement is used?

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