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Resistant *E. coli* isolated from smallholder pig farms in Lira, Uganda

Kristin Selling

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Kristin Selling

Supervisor: *Magdalena Jacobson, Department of Clinical Sciences*

Assistant Supervisor: *Kokas Ikwap, College of Veterinary Medicine and Biosecurity, Makerere University*
Assistant Supervisor: *Ingrid Hansson, Department of Biomedical Sciences and Veterinary Public Health*
Assistant Supervisor: *Lise-Lotte Fernström, Department of Biomedical Sciences and Veterinary Public Health*
Assistant Supervisor: *Elin Gertzell, Department of Clinical Sciences*

Examiner: *Per Wallgren, Department of Clinical Sciences*

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SUMMARY

Uganda is an East African country with a fast-growing population and has one of the world's lowest gross domestic product per capita. Most of the population live in rural areas and agriculture is the country's most important source of income. There are about 4 million pigs in Uganda, most are kept at smallholder farms, where they are an important source of income, and are most often sold to traders when in need of money. Only 5% of the pig-keepers in Uganda hold the pigs for own consumption. The traders often sell the pigs to slaughter slabs were the hygiene is suboptimal.

Antibiotic resistance is one of the great global concerns of our time. Bacterial resistance can be both natural and acquired and can be transferred in-between bacteria. The acquired resistance is driven by natural selection and use of antibiotic drugs. Therefore, the use of antibiotics must be controlled, especially the treatment of animals. In some places the misuse of antibiotics is vast, and it is sometimes used prophylactically and as growth promotors. The use of antibiotics is uncontrolled, and drugs can be bought over the counter in many places around the world, Uganda is an example of such a place. Moreover, bacteria carrying genes for antibiotic resistance can spread from animals to humans via the food-chain. In some countries, including Sweden, there are national surveillance programs to observe the use of antibiotics and the resistance in the population. One strategy of surveillance is to use indicator-bacteria, investigating resistance in a normal intestinal bacterium such as *Escherichia (E.) coli* to indicate the overall resistance and selective pressure in a population.

In this study, pigs from twenty smallholder farms around Lira in northern Uganda, were sampled and investigated regarding the presence of antibiotic resistance, using *E. coli* as an indicator bacterium. The farmers were also interviewed on their use of antibiotic drugs. The results showed that 67% of the farms had treated their pigs with antibiotics during the last year. Out of the 53 samples, 88% were resistant to sulfamethoxazole, 54% to tetracycline and 17% to trimethoprim. Further, 19% were multidrug-resistant, *e.g.* resistant to three or more antibiotic classes.

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ABBREVIATIONS

ASF: African Swine Fever
CAMHB: cation adjusted Müller Hinton broth
CLED-agar: Cysteine Lactose Electrolyte Deficient-agar
ECOFF-value: Epidemiological cut off-value
EUCAST: European Committee on Antimicrobial Susceptibility Testing
FAOSTAT: Food and Agricultural Organization Statistics Division
HGT: horizontal gene transfer
ILRI: International Livestock Research Institute
MIC: minimum inhibitory concentration
SIDA: Swedish International Development Cooperation Agency
SLU: Swedish University of Agricultural Sciences
SVA: National Veterinary Institute (Sweden)
UBOS: Ugandan Bureau of Statistics
WHO: World Health Organization

INTRODUCTION

Winston Churchill once called Uganda the Pearl of Africa. It is an East African country on the northern shore of Lake Victoria that is rich in natural resources, there is wildlife and beautiful nature. The climate is pleasant and beneficial for agriculture which is the country's most important source of income. Even so, it is a low-income country and has a young and fast-growing population. It is common for people in rural areas to keep livestock, and even though goats are most popular, there are around 4 million pigs in Uganda, most of them being held at smallholder farms (UBOS, 2008; UBOS, 2018).

Antibiotic resistance is one of the major global issues of our time and has been acknowledged as such by the United Nations (PRESS RELEASE: High-Level Meeting on Antimicrobial Resistance, 2016). Some bacteria harbor natural resistance to antibiotics, whereas others can acquire resistance from the environment or from other bacteria, making previously susceptible bacteria resistant to the drug (Munita & Arias, 2016). The acquired resistance is mainly driven by the use of antibiotic substances, this being a great concern since in some areas the misuse of antibiotics is extensive, using valuable drugs without accurate diagnostics, as prophylactics or even to promote growth in food-producing animals. Antibiotics are used inappropriately all over the world, generating even more resistance. The development of antibiotic resistance is under surveillance in some areas, for example in the European Union (EU summary report, 2017). However, there is no such program in Uganda. In the surveillance programs, resistance in *Escherichia (E.) coli* may indicate the overall resistance and selective pressure on the bacteria in a population, therefore *e.g. E. coli* can be used as an indicator-bacterium. *E. coli* is a commensal in the gut of all warm-blooded species with the ability to share its genes of resistance with other bacteria, including pathogens. If pathogens acquire antibiotic resistance, problems will arise where we no longer have the means to fight infections that we previously have been able to treat.

The aim of this minor field study was to investigate the occurrence of antibiotic resistance in *E. coli* in smallholder pig-farms around Lira in Uganda. The farmers were interviewed regarding their use of antibiotics to investigate any relation between the consumption and potential resistance. Furthermore, the aim was to gather understanding and experience regarding animal husbandry, field work and veterinary medicine in a low-income country.

LITERATURE REVIEW

Uganda

Uganda is situated in east Africa and has one of the world's youngest population with a median age of 15.9 years (CIA, 2019). It is a very fertile country with a beneficial climate and agriculture is the main occupation, engaging over 70% of the population. There is approximately 41 million people living in Uganda, and about 75% lives in rural areas. Most people farm their own parcel of land, cultivating what the family consumes and, in some cases, additional produce to sell at markets. Uganda has one of the lowest gross domestic product per capita in the world (CIA, 2019), and 41% of the population is malnourished (FAOSTAT, 2019).

Pig-keeping in Uganda

Over 70% of Uganda's population keep livestock such as goats, cattle, sheep and pigs, goats being the most common. The pig population has increased considerably during the last 30 years. There are roughly 4 million pigs in Uganda (UBOS, 2018) and most pig-holding farms have less than ten pigs, the vast majority having less than five pigs (UBOS, 2008). However, there are some large pig producers with an intensive farming system, mostly in urban areas around Kampala. The pigs of Uganda are regularly mixed breeds between Landrace and Large White that were imported in the 1960's, and the indigenous black pig (Tatwangire, 2014).

Consumption of pork in the households is quite rare, but the demand for the meat at so called 'pork joints' (small restaurants that serve pork) is growing, and so is the demand from restaurants and hotels. Of the farmers, 95% stated that they kept pigs as a financial source, and only 5% kept pigs for their own consumption (Muhanguzi *et al.*, 2012). The pig industry in Uganda is small but the country has a good possibility to become self-sufficient in pork products. Pork is a good source of nutrients in a country where malnutrition is of immense concern. The pig industry is a good income for people in rural areas, farmers keep pigs as savings, selling them when they need money for school fees and other expenses (Atherstone *et al.,* 2019). Further, it is a quite common livestock for women to keep, rending it a way forward for gender equality and poverty reduction. Pigs are also a relatively easy livestock to keep, since they are fast growing and has a high reproduction rate. They are often fed on maize, cassava and other crops that are usually grown in the households, however, this also causes problems since the pig feed competes with the recourses for human food (Dione *et al.*, 2014; Tatwangire, 2014).

Pigs and other livestock are grazed alongside crop-growing fields in Uganda, both in rural and urban areas. Pigs are kept free-ranging, tethered or in pens. The floor in the pens might be raised or made out of concrete, but soil floor is also common (Dione *et al.*, 2014). Soil floor as well as the keeping of pigs free-ranging or tethered causes hygiene problems, since it is hard or impossible to keep clean. This is a perfect environment for the growth and survival of bacteria and parasites. Keeping pigs outdoors, with possible contact with other pigs and wildlife also increase the risk of diseases such as African Swine Fever. In urban areas there are some intensive production systems, with more advanced housing and feed (Muhanguzi *et al.*, 2012; Ouma *et al.,* 2015). Most farmers do not have their own boar; it is common to use a village boar. This causes problems with spread of disease and inbreeding (Tatwangire, 2014).

There is a raising concern in the public about food safety in the country, and hygiene conditions are poor. Pigs are often sold from the small-holder farms or from markets to traders whom bring the pigs to slaughter (Atherstone *et al.,* 2019). In urban areas, there are a few larger slaughterhouses with inconsequential animal and meat inspection. However, most meat is not inspected at slaughter, especially the meat that are sold at local, informal slaughter slabs. Pork is sold to consumers on the street, in the markets, in the supermarkets and ready-to-eat at pork joints. Cooled storage and cooled transport of meat is very rare, and the hygiene during slaughter is poor. This is a serious potential health concern in the food chain, for example, an infection with the porcine tapeworm may cause cysticercosis, a zoonosis that can cause myositis and neurological symptoms in humans (Muhanguzi *et al.,* 2012).

Diseases

One of the most feared diseases that pig keepers in Uganda face is African Swine Fever. There are outbreaks in all pig-keeping areas, all around the year. An outbreak always has a great impact on the farmers' economy, either if all the pigs deceases, and people will immediately sell their live pigs but at low prices. There is also a risk of losing good-quality breeding animals. Other common health issues are the parasites, both internal and external, diarrhea and coughing. These diseases also causes economic challenges since the growth rate of the pigs decreases, and of course have an impact on animal welfare. Veterinary services are expensive and there is a lack of professionals in rural areas. Treatment of animals by the farmers themselves is common, drugs are easily accessible over the counter, and traditional medicine is also common (Dione *et al.,* 2014; Muhanguzi *et al.,* 2012). There are also problems with fake drugs, for example there are people selling a vaccine to prevent ASF, although no such vaccine is available today (Ouma *et al.,* 2015; Tatwangire, 2014). According to Okello *et al.* (2014) vaccination in piggeries in Uganda is rare.

Antibiotic usage

Inappropriate use of antibiotics in livestock, such as prophylactic use of antibiotics in healthy animals and ignorant treatments, are common in low-income countries. The use of antibiotics such as penicillin, streptomycin and tetracycline are most commonly used in livestock, for both prevention and treatment of bacterial infections (Van Puyvelde *et al.,* 2018). This causes the presence of residues of the drugs in animal-based food such as milk, eggs and meat (Basulira *et al.,* 2019). Tetracyclines, penicillin and penicillin combined with streptomycin (Pen-strep) are the antibiotics that are most commonly used, since they are relatively cheap and easily available over the counter (Van Puyvelde *et al.,* 2018). Tetracycline is a broad-spectrum antibiotic and is also used as a growth promoter, a usage that is forbidden in Sweden since 1986 and in Europe since 1996 (Bengtsson & Greko, 2014). This usage is especially common in poultry production and Kabiswa *et al.* (2018) found in their study from Uganda a high prevalence of resistance, in particular, 87% of the *E. coli* strains were resistant to tetracycline.

In one study from Uganda the farmers were interviewed on their use of antibiotics and it was found that the use of penicillin could be related to the ampicillin-resistance (Okubo *et al.,* 2019). It was common to treat livestock with penicillin and sometimes with streptomycin, as an injection or as a spray used on wounds in cattle and pigs. Oxytetracycline was along with penicillin the most commonly used antibiotics. Some farmers also used sulfonamides, with or without trimethoprim. It was also common to give sulfonamides or oxytetracycline in the feed or water to laying hens. In line with this prophylactic use of penicillin and tetracycline is common. According to this study no cephalosporins or carbapenems were used, and the authors concluded that it was too expensive (Okubo *et al.*, 2019).

Pathogenic *Escherichia coli*

E. coli is a commensal bacterium, possessing many various properties. Most strains of *E. coli* are non-pathogenic and are part of the normal intestinal bacterial flora, however some strains are pathogenic and might cause disease in humans and animals. Strains of *E. coli* possess a range of different virulence factors and are divided into different pathotypes accordingly. In pigs, the main pathotypes are VTEC (verotoxin producing *E. coli)* causing edema disease and ETEC (enterotoxigenic *E. coli*) causing neonatal and post weaning diarrhea (VetBact, 2019). These diseases can be combatted by prophylactic methods such as adequate colostrum supply to newborn piglets and gradual changes of feed. There is also vaccines available for neonatal diarrhea and for edema disease. It is not recommended to treat piglets that is already presenting clinical signs of edema disease with antibiotics, but one might consider treating pigs in the same pen that do not have any clinical signs to prevent them from disease (SVA, 2018a; SVA, 2018b).

In humans, there are a few pathogenic strains of *E. coli* causing different diseases including enteric infections and septicemia. Enterohemorrhagic *E. coli* (EHEC) causes food poisoning, enterohemorrhagic diarrhea and hemolytic uremic syndrome (HUS). Urinary tract infections are also common (VetBact, 2019).

The importance of antibiotic resistance

In a study on post-weaning diarrhea caused by enterotoxigenic *E. coli* (ETEC) in Uganda, high levels of antibiotic resistance and multi-drug resistance were found among the isolated strains (Okello *et al.*, 2015). Further, antibiotic prophylaxis was commonly used in the area. The study concluded that post-weaning diarrhea is widespread in Central Uganda but since there is a vast use of prophylactic antibiotics, clinical outbreaks are uncommon. In 142 isolates, 100% were resistant to penicillin and erythromycin and 68% were resistant to tetracycline and nitrofurantoin, 41% to ampicillin and 33% to cotrimoxazole. Furthermore, additionally tested antibiotics e.g. chloramphenicol and nalidixic acid demonstrated lower percentages of resistance of 9% each, however all strains were susceptible to ciprofloxacin. Widespread multi-drug resistance was also found among the isolates (Okello *et al.*, 2015).

An alarming study using *E. coli* as an indicator bacterium in wildlife used as sentinel animals in Botswana, showed the impact of antibiotic resistance in humans and demonstrate how difficult it is to control (Jobbins & Alexander, 2015). Of the *E.* coli isolated in fecal samples from wild animals, 13.3% of the isolates were multidrug-resistant and 41.1% of the isolates were resistant to one or two out of ten antibiotics. The resistance was compared to human clinical and environmental samples and a corresponding spectrum in the different sample units, including resistance to ampicillin, doxycycline, streptomycin, tetracycline or trimethoprim-sulfamethoxazole, was identified. Resistance was more frequent in carnivores, water-associated species and in urban areas, showing that lifestyle, position in the food-chain, and imminence to humans, had an impact. Furthermore, antibiotic resistance transmitted by bacteria in water, could be an introduction of antibiotic resistance to new populations. Water and sediment may as well as animals serve as a source of resistant *E. coli*. However, the study did not include any molecular analyses and thus other sources of resistance could not be excluded (Jobbins & Alexander, 2015).

Not to be forgotten, antibiotic resistance in animals does not only have an impact on human health through the food chain, but it also has an impact on animal welfare. Treatment of bacterial infections in animals will in the future perhaps be ineffective, especially since any new antibiotics discovered, will be reserved for human medicine. This leads to suffering for the animals as individuals but also affects production and the farmers' financial situation, as well as the consumer, since food will be more expensive and possibly less in quantity (Bengtsson & Greko, 2014; Schmithausen *et al.,* 2018).

Mechanisms of antibiotic resistance

The mechanism of antibiotic resistance in bacteria is an expected strategy of survival. The theory of "the survival of the fittest" is apt since if being exposed to antibiotics, the bacteria still susceptible to antibiotics will be erased whereas bacteria carrying genes for mechanisms of resistance will survive and spread, making antibiotics useless. The bacterial genome is flexible and is inclined to incorporate genes that are favorable for their survival. There are two basic strategies of bacteria to modify their genes in response to the treatment of antibiotics; 1) Mutations in specific genes that associates with the approach of the antibiotics, basically decreasing the uptake of the drug, increasing the efflux or modify the target of the drug *e.g.* decreasing the affinity. Such mutations are often not beneficial for the bacteria's fitness; thus, they are only maintained when needed, and the expression of resistant genes will be down-regulated when antibiotics are no longer present. However, the genes are still present, even though they are not expressed when not needed. 2) Horizontal gene transfer (HGT) is a mechanism where bacteria obtain foreign DNA including mechanisms of resistance, mostly from products in their environment. There are three different main mechanisms of HTG; transformation, which is when naked DNA is incorporated in the bacteria; transduction, were a phage (bacterial virus) mediate the transfer of DNA; and conjugation, were the cells are in direct contact with each other and transfer the genes using mobile genetics elements such as plasmids (Munita & Arias, 2016). Transformation often occurs after cell lysis when there is DNA in the environment that the bacteria may pick up. Plasmids contain DNA that is separated from the chromosome and contains genes that may encode for antibiotic resistance, the conjugation then occurs, and the plasmid is transferred in-between bacteria via sex pili, and both bacteria end up with a copy of the plasmid. Conjugation is most common in Gram-negative bacteria but also appears less frequently in Gram-positive bacteria (McManus, 1997). Multidrug resistance implies that a bacterium is resistant to three or more antibiotic classes, according to the Swedish surveillance program (Swedres-Swarm, 2018). Resistance in bacteria can be either natural or acquired, though natural resistance may be less central in clinical situations. Acquired resistance on the other hand is significant since it is driven by the use, and more importantly, misuse of antibiotics, and is developed in bacteria that was previously susceptible to it (Munita & Arias, 2016).

The mechanisms by which the antibiotic drugs exert their mode of action includes interference with the synthesis of the cell wall (penicillin, cephalosporins, carbapenems), proteins (macrolides, chloramphenicol, clindamycin, aminoglycosides, tetracyclines) or nucleic acids (fluoroquinolones) in the bacteria, also disturbing metabolic pathways (sulfonamides, trimethoprim) or destroying the membrane structures (polymyxins). Some bacteria produce enzymes that destroy antibiotics. As an example, the action of penicillin is through the component beta-lactam that interfere with the cell wall of bacteria. However, some bacteria produce the enzyme beta-lactamase that hydrolyze beta-lactams and make the antibiotic useless (Tenover, 2006).

Antibiotic resistance can be transmitted in-between bacteria with plasmids and other encoded transferable genetic elements. *E. coli* in the normal intestinal bacterial flora can receive these genes and act as a reservoir of resistance (Okubo *et al.,* 2019). For example the genes for tetra-cycline resistance are located on such mobile genes and are easily transmitted by *E. coli* (Kabiswa *et al.,* 2018). The common strategy developed by bacteria to circumvent the action of tetracycline is to produce efflux pumps that reject the drug from the cell (Munita & Arias, 2016).

Escherichia coli as an indicator bacterium of antibiotic resistance

Antibiotic resistance is when bacteria develop resistance mechanisms that are effective against different types of antibiotics to which they previously have been susceptible. This insusceptibility can derive from natural selection but is also driven by the inappropriate use of antibiotics, both in human and veterinary medicine. Antibiotic resistance is a major global health issue, since it turns infectious diseases untreatable or prolongs the recovery (Kirbis & Krizman, 2015). In livestock, antibiotic-resistant bacteria can be transmitted between human and animals directly, by environmental routes such as water but also through the food-chain, since it can easily contaminate food products during slaughter. Several countries have a surveillance program to monitor the evolvement of antibiotic resistance and it is mandatory within the European Union. One of the surveillance strategies is to use so-called indicator bacteria, *i.e.* a commensal bacterium that is analyzed for the presence of antibiotic resistance. In food producing animals, *E. coli* is commonly used as an indicator bacterium in fattening pigs at slaughter (EU summary report, 2017). *E. coli* is a Gram-negative commensal in the intestinal bacterial flora of all warm-blooded species and is recommended as a sentinel bacterium. Most *E. coli* strains are apathogenic but there are also some pathotypes that can be pathogenic for both humans and animals, even though most bacteria in the enteric flora are unlikely to cause disease. *E. coli* carrying resistance genes can colonize in the intestines of food-producing animals such as pigs and act as a reservoir and be transmitted to humans through the food chain. Viewing *E. coli* in the intestinal flora of healthy animals as an indicator-bacterium will provide a picture of the overall resistance and selective pressure of the antibiotics used in the population. This indicates what humans could be exposed to within the food-chain. Therefore, it is valuable and important to monitor *E. coli* as it can serve as a reservoir for resistance genes in the intestinal flora. Resistant genes may be transferred to other bacteria including pathogens, making them resistant to antibiotics and in the end making the antibiotics useless (Swedres-Swarm, 2018).

However, there is no national surveillance program for antibiotic resistance in Uganda (Van Puyvelde *et al.*, 2017).

A study from Uganda used *E. coli* as an indicator bacterium to investigate antimicrobial resistance in livestock (pigs, cattle, goats, layers). In 130 fresh fecal samples, *E. coli* resistant to ampicillin (44,8%), tetracycline (97,0%) and sulfamethoxazole-trimethoprim (56,7%) were demonstrated (Okubo *et al.*, 2019).

Another study on dairy cattle was performed in Wakiso District in Uganda, using *E. coli* as an indicator bacterium, showed that 21% of the isolates were resistant to one antibiotic, and 7% were multidrug-resistant (Ball *et al.,* 2019). Resistance towards tetracycline, sulfamethoxazole and streptomycin was the most prevalent. Tetracycline was the antibiotic mostly used, both prophylactically and for treatment. They also demonstrated that the resistant strains in Uganda had low genetic diversity. People often live close to their animals and share water source, therefore bacteria and resistance can easily be spread (Ball *et al.*, 2019).

Prevention of antibiotic resistance

Healthy animals do not need antibiotics. When antibiotics are used prophylactically or as a growth promoter, the selective pressure increases. This prophylactic use should be replaced by management measures that can be taken into consideration to improve health and reduce the need of antibiotics, for example avoid the mixing of animals from different groups, age or farms. Other examples include to change the practice of keeping many animals in a small confinement or not cleaning the housing properly, that will increase the infectious load of commensals such as *E. coli* that is often found in manure and wastewater. Furthermore, the implementation of health-monitoring programs *i.e.* heard health management, is urgently advocated (Bengtsson & Greko, 2014).

Globally, the motives for the use and provision of antibiotics varies. Some countries, such as Sweden, has a restrictive antibiotics policy. According to the Swedish Board of Agriculture´s regulations regarding medical products and their usage (SJVFS 2019:32, saknr. D9), antibiotics are only to be obtained following the prescription from a veterinarian. Having such a regulation in place is crucial for saving our existing antibiotics and to diminish the risk of occurrence of non-pathogenic resistant bacteria (Schmithausen *et al.,* 2018). In countries where farmers can buy antibiotics over the counter without veterinary prescription, there is an increased risk of misuse of antibiotics and antibiotic resistance (Jobbins & Alexander, 2015; Okubo *et al.,* 2019). The fact that there is low access to qualified veterinary health care and poor awareness in low-income countries contributes to the situation (Van Puyvelde *et al.,* 2017).

MATERIAL AND METHODS

This study was performed in collaboration with Makerere University in Kampala, Uganda, within an International Livestock Research Institute (IRLI)-project on animal husbandry and heard health management in Lira in Uganda. This is an ongoing licentiate project including twenty smallholder pig farms. Under the same project another study on occurrence of MRSA was carried out by another student (Dahlin, 2020). The project was primarily financed by a Minor Field Scholarship from SIDA (Swedish International Development Cooperation Agency).

The twenty farms included in the study housed 1-150 pigs and 1-10 samples were collected from each farm depending on the number of pigs they held at the time of the visit. Samples were taken from the rectum using a swab (Figure 1) transported in Amies' transport medium with charcoal (Copan Diagnostics Inc., Brescia, Italy) in a cooling bag to the field laboratory. During the visit, the farmers were interviewed on their use of antibiotics (Appendix 1) Since all of the farmers were included in a project on animal husbandry, all had a journal where they were supposed to note all treatments, and these were reviewed during the visit to complement the farmers answers.

Figure 1. *Sample collected from rectum (Elin Gertzell, 2019).*

At the field laboratory, the samples were initially cultured on CLED-agar (cysteine lactose electrolyte deficient-agar) (SLU, Uppsala, Sweden), a selective electrolyte (salt)-deficient medium, which prevent the swarming of *Proteus* spp. and turns yellow if the bacteria ferments lactose (Figure 2). The plates were incubated at 37°C for 24 hours and thereafter, five suspected *E. coli* colonies (yellow, opaque colonies that sometimes had a deeper yellow center) from each plate were pure-cultured on horse-blood agar plates (SVA, Uppsala, Sweden), and incubated at 37°C for 24 hours. Confirmation of *E. coli* was performed by morphology (greyish colonies with no hemolysis or beta hemolysis), oxidase test (neg), spot indol test (pos) and potassium hydroxide test (neg). If the colony proved to be an *E. coli*, it was transferred to a new swab of Amies' transport medium with charcoal (Copan Diagnostics Inc., Brescia, Italy), and stored in the refrigerator before being transferred the laboratory at Makerere.

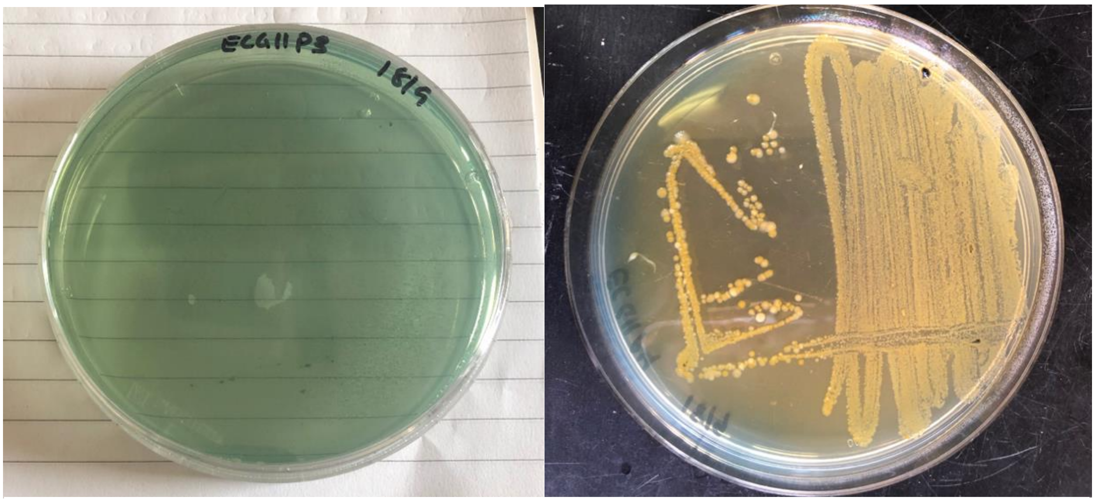

Figure 2. *a) CLED-agar before culturing b) CLED-agar with growth of lactose-fermenting bacteria (author 2019).*

At the laboratory, the bacteria were re-cultivated on horse-blood agar plates and incubated at 37°C for 24 hours. One suspected colony was pure-cultured once more on horse-blood agar plates (SVA, Uppsala, Sweden) and incubated at 37°C for 24 hours.

Testing for antibacterial resistance was initiated by CAMHB (cation adjusted Müller Hinton broth) (SVA, Uppsala, Sweden) being poured into falcon tubes, one containing 5 mL and one containing 10 mL per pure-cultured colony, 1 µL (one full inoculation loop) material from the agar-plate was transferred into the 5 mL tube and was vortexed. Thereafter, 10 µL from this tube was transferred to the tube containing 10 mL of CAMHB vortexed and incubated at 37°C for 1 hour and 50 minutes. From this broth, 50 µL was transferred to each of the wells in a EUSEV micro-dilution plate (ThermoFisher Sensititre™, Massachusetts, USA; Figure 4) with 96 wells containing 14 different antibiotics in increasing concentration. The plates were incubated at 37°C for 18 hours. The strain ATCC 25922 was similarly analyzed as a positive control. Confirmation of the density of the inoculum was performed, and 10 µL from one random 10 mL broth-tube with bacteria was transferred to 10 mL 0.86 – 0.90% sodium chloride. The samples were vortexed and 100 µL were spread on a horse-blood agar-plate and incubated at 37°C for 18 hours. Simultaneously, each sample was cultured on horse blood agar plates and incubated at 37°C for 18 hours to confirm the purity. The results of the MIC-dilution plates were interpreted according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) epidemiological cut-off values for resistance (ECOFF).

RESULTS

All but one of the 20 visited farms kept pigs at the time of our visit, hence pigs from 19 different farms were sampled. Two farms were larger, keeping 98 and 126 pigs, respectively. The remaining 17 farms were smaller and kept 1-30 pigs (median 9.7 pigs). One to ten samples were collected at each farm, in total 53 samples, one pig per sample.

An interview regarding the use of antibiotics was performed on each of the 19 farms. At the farms, the main caretaker was interviewed if present, and in other cases a family member or the household head was interviewed. Despite the assistance of an interpreter, not all questions could be answered at all farms. The questions (Appendix 1) addressed the antibiotic treatment on the farm during the last year and 17 (89%) of the farms had treated the pigs. Six of these farmers did not have any knowledge on the drug used. Most of the treatments were performed by a veterinarian or a para-veterinarian, but four farmers had drugs at home and treated the pigs themselves. These farmers also performed routine treatments, besides treating the pigs when they showed clinical signs. The remaining farmers only treated the pigs when they showed signs of disease. Clinical signs in treated pigs included wounds/injuries, skin lesions (spots, red skin, lesions, loss of hair), diarrhea, loss of weight or appetite, decreased demeanor, ectoparasites (lice or mites), fever, coughing and shivers. Oxytetracycline was the most commonly used antibiotic (10 farms), but four farmers used penicillin-streptomycin as well. Single farmers also treated the pigs with sulfonamides or with macrolides (tylosine; Table 1). If a veterinarian had been treating the pigs, the veterinarian also brought the drug, and returned for additional treatments if needed. The farmers that treated the pigs themselves bought the drugs at the local pharmacy (Figure 3).

Figure 3. *The medical supply at one of the farms, including Oxytetracycline, Pen-strep, Alamycin-spray and dewormers.*

E. coli was found in 52 of 53 samples. In the analyses of antibiotic resistance and comparing to the EUCASTs values of MIC (minimum inhibitory concentration), 88% of the isolates were resistant to sulfamethoxazole, 54% to tetracycline and 17% to trimethoprim. Further, 12% were resistant to ampicillin, 8% to cefotaxime and ciprofloxacin, 6% to chloramphenicol, 4% to gentamicin and azithromycin, and 2% to colistin and ceftazidime (Table 2). No resistance was discovered against meropenem, nalidixic acid or tigecycline. Ten (19%) out of the 52 isolates were resistant to three or more different classes of antibiotics, which is the Swedish definition of multidrug-resistant (Swedres-Swarm, 2018). One of this 10 isolates was resistant against seven different classes and another sample was resistant against eight classes of antibiotics (Table 3).

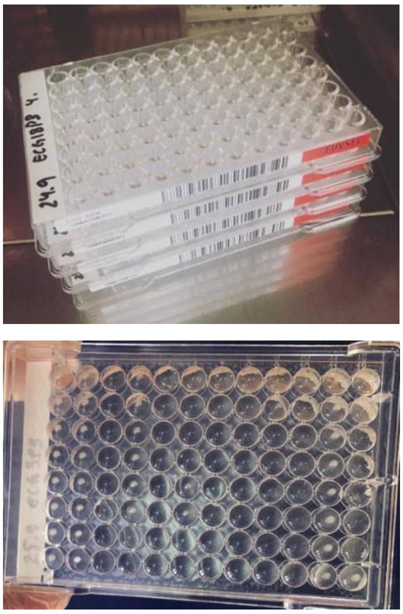

Figure 4. *Mic-dilution plates (author, 2019).*

Table 1. *The farms stated use of antibiotics during the last year (2018-2019) in 20 farms in Lira, Uganda*

Antibiotics	**Oxytetracycline**	**Penicillin-Streptomycin**	**Sulfonamide**	**Tylosine**	**Unspecified antibiotic**
Farm 1					X
Farm 2	X				
Farm 3					X
Farm 4					X
Farm 5	X	X			
Farm 6					
Farm 7	X				
Farm 8					X
Farm 9					X
Farm 10	X				
Farm 11	X				
Farm 12	X				
Farm 13		X			
Farm 14	X				
Farm 15	X			X	
Farm 16	X	X			
Farm 18	X	X	X		
Farm 19					X
Farm 20					

The ten multi-resistant *E. coli* strains were found on five farms. All of these farms had treated their pigs with antibiotics during the last year. Four of these farmers routinely treated the pigs, in addition to treating those pigs that displayed signs of disease. Signs that indicated treatment on the farms included diarrhea and skin lesions, but one farmer have used antibiotics as he believed that it would make the pigs grow faster. Another farmer thought that antibiotics would improve the pig's health. Three farms had used oxytetracycline, two had used penicillin-streptomycin and one sulfonamide, whereas three farms did not know what drugs had been used. Resistance against tetracycline was found in 28 samples, and 19 of them originated from farms that stated to have had treated their pigs with oxytetracycline during the last year.

Table 2. *Number of resistant samples, from a total of 52 samples. ECOFF (epidemiological cut off)-values are given in μg/mL*

Antibiotics	ECOFF values	Number of Resistant Isolates	Percentage Resistant Isolates
Sulfamethoxazole (SMX)	>64	46	88%
Trimethoprim (TMP)	>2	9	17%
Ciprofloxacin (CIP)	>0,06	4	8%
Tetracycline (TET)	>8	28	54%
Meropenem (MERO)	>0,12	0	0%
Azitromycin (AZI)	>16	2	4%
Nalidixic acid (NAL)	>16	0	0%
Cefotaxime (FOT)	>0,25	4	8%
Tigecycline (TGC)	>0,5	3	6%
Ceftazidime (TAZ)	>0,5	0	0%
Colistin (COL)	>2	1	2%
Ampicillin (AMP)	>8	1	2%
Gentamicin (GEN)	>2	6	12%

Table 3. *Distribution of MIC values (µg/mL) among* E. coli*-samples. Epidemiological cut off-values are taken from EUCAST or Swedres-Svarmpat (2018). Abbreviations: SMX=sulfamethoxazole, TMP=trimethoprim, CIP=ciprofloxacin, TET=tetracycline, MERO=meropenem, AZI=axitromycin, NAL=nalidixid acid, FOT=cefotaxime, CHL=chloramphenicol, TGC=tigecycline, TAZ=ceftazidime, COL=colistin, AMP=ampicillin, GEN=gentamicin, Multires.= multidrug-resistant* e.g. *resistant towards three or more different antibiotic classes*

Antibiotics		SMX	TMP	CIP	TET	MERO	AZI	NAL	FOT	CHL	TGC	TAZ	COL	AMP	GEN	Multires.
ECOFF		**>64**	**>2**	**>0,06**	**>8**	**>0,12**	**>16**	**>16**	**>0,25**	**>16**	**>0,5**	**>0,5**	**>2**	**>8**	**>2**	**YES/NO**
Farm 1	**Sample 1**	>1024	<0,25	<0,015	<2	<0,03	X4	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 2	>1024	<0,25	<0,015	X32	<0,03	X8	<4	<0,25	X16	<0,25	X1	<1	X2	<0,5	YES
Farm 2	**Sample 1**	>1024	X0,5	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X4	<0,5	NO
	Sample 2	>1024	X0,5	<0,015	<2	<0,03	X4	<4	<0,25	<8	<0,25	<0,5	<1	X4	<0,5	NO
	Sample 3	X32	<0,25	<0,015	<2	<0,03	X4	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
Farm 3	**Sample 1**	<8	<0,25	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	X2	NO
	Sample 2	>1024	>32	<0,015	X32	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	>64	<0,5	YES
	Sample 3	>1024	>32	<0,015	>64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	>64	<0,5	YES
Farm 4	**Sample 1**	>1024	<0,25	<0,015	X8	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X4	X1	NO
Farm 5	**Sample 1**	>1024	X1	<0,015	>64	<0,03	X8	<4	<0,25	X16	<0,25	<0,5	<1	X2	X4	YES
	Sample 2	>1024	<0,25	<0,015	>64	<0,03	X4	<4	<0,25	<8	<0,25	<0,5	<1	X4	X1	NO
	Sample 3	>1024	<0,25	<0,015	<2	X0,06	X8	<4	<0,25	<8	<0,25	<0,5	<1	X8	X2	NO
	Sample 4	>1024	X4	X0,03	X64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	X2	NO
	Sample 5	>1024	<0,25	<0,015	>64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X4	X2	NO
	Sample 6	>1024	<0,25	<0,015	>64	<0,03	X4	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 7	>1024	X0,5	<0,015	>64	<0,03	X16	<4	<0,25	<8	X0,5	<0,5	<1	>64	X2	YES
	Sample 8	>1024	<0,25	<0,015	>64	<0,03	X4	<4	X1	<8	X0,5	<0,5	<1	X4	X2	YES
	Sample 9	>1024	>32	X1	>64	<0,03	X8	X16	X0,5	X32	<0,25	<0,5	<1	X64	X16	YES
	Sample 10	>1024	<0,25	<0,015	>64	X0,06	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
Farm 6	**Sample 1**	<8	<0,25	<0,015	X8	<0,03	<2	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 2	>1024	X0,5	<0,015	X8	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X8	<0,5	NO
Farm 7	**Sample 1**	>1024	X2	<0,015	X4	<0,03	>64	<4	<0,25	<8	<0,25	<0,5	<1	X8	X1	NO
Farm 8	**Sample 1**	>1024	X0,5	<0,015	<2	<0,03	X8	<4	X0,5	<8	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 2	>1024	>32	X0,5	>64	<0,03	X8	X8	<0,25	<8	<0,25	<0,5	<1	>64	X1	YES
	Sample 3	>1024	<0,25	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	<1	X1	NO
Farm 9	**Sample 1**	>1024	X0,5	<0,015	<2	<0,03	X32	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 2	>1024	X0,5	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 3	>1024	X1	<0,015	X8	<0,03	X16	<4	<0,25	<8	<0,25	<0,5	<1	X4	X1	NO

Antibiotics		SMX	TMP	CIP	TET	MERO	AZI	NAL	FOT	CHL	TGC	TAZ	COL	AMP	GEN	Multires.
ECOFF		>64	>2	>0,06	>8	>0,12	>16	>16	>0,25	>16	>0,5	>0,5	>2	>8	>2	Yes/No
Farm 10	Sample 1	>1024	X1	<0,015	>64	<0,03	X16	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 2	<8	<0,25	<0,015	<2	<0,03	<2	<4	<0,25	<8	<0,25	<0,5	<1	<1	<0,5	NO
	Sample 3	*No E. coli*														
	Sample 4	>1024	>32	<0,015	>64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
Farm 11	Sample 1	>1024	<0,25	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 2	X16	X0,5	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X4	X1	NO
	Sample 3	>1024	X0,5	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 4	>1024	>32	X0,03	>64	<0,03	X16	<4	<0,25	<8	<0,25	<0,5	<1	X4	X1	NO
Farm 12	Sample 1	>1024	>32	X0,06	>64	<0,03	X16	<4	<0,25	X16	X0,5	<0,5	<1	X4	<0,5	NO
Farm 13	Sample 1	>1024	<0,25	<0,015	>64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 2	>1024	X0,5	<0,015	>64	<0,03	X4	<4	<0,25	X16	<0,25	<0,5	<1	X8	X1	NO
	Sample 3	>1024	<0,25	X0,06	>64	<0,03	X16	<4	<0,25	<8	X0,5	<0,5	<1	X4	<0,5	NO
Farm 14	Sample 1	>1024	X1	X0,03	>64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
Farm 15	Sample 1	>1024	<0,25	<0,015	<2	<0,03	X4	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 2	>1024	<0,25	<0,015	>64	<0,03	X8	<4	<0,25	X16	<0,25	<0,5	<1	X2	X1	NO
Farm 16	Sample 1	>1024	>32	X0,12	X32	<0,03	X8	<4	<0,25	>128	<0,25	<0,5	<1	>64	X1	YES
	Sample 2	>1024	X0,5	<0,015	<2	<0,03	X4	<4	<0,25	<8	X0,5	<0,5	<1	X8	<0,5	NO
	Sample 3	>1024	<0,25	X0,03	X64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
Farm 18	Sample 1	>1024	<0,25	<0,015	<2	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 2	>1024	<0,25	<0,015	X64	<0,03	X8	<4	<0,25	X16	<0,25	<0,5	<1	X2	<0,5	NO
	Sample 3	>1024	<0,25	<0,015	>64	<0,03	X8	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 4	>1024	<0,25	<0,015	X64	<0,03	<2	<4	<0,25	<8	<0,25	<0,5	<1	X2	X1	NO
	Sample 5	<8	X0,5	X0,25	<2	<0,03	X4	<4	X0,5	<8	<0,25	<0,5	X8	X2	<0,5	YES
Farm 19	Sample 1	>1024	<0,25	<0,015	X64	<0,03	X8	X8	<0,25	<8	<0,25	<0,5	<1	X8	<0,5	NO
Farm 20	Sample 1	>1024	<0,25	<0,015	<2	<0,03	X4	<4	<0,25	X32	<0,25	<0,5	<1	X2	<0,5	NO

DISCUSSION

Within this study, 54% of the isolates were resistant against tetracycline, the same class of antibiotic as oxytetracycline, which was the most commonly used antibiotic on the farms. This in accordance with Okello *et al.* (2014) who found 68% resistance towards tetracycline in their study in Ugandan piggeries. As a comparison, in the last report from Swedres-Swarm (2018), 9% of the *E. coli* from slaughtered pigs in Sweden were resistant to tetracycline. In Europe, the overall resistance in slaughtered pigs was 52.1% in 2017, the resistance varying among the different countries (EU summary report, 2017). Even though it is interesting to compare the results with statistics from Sweden, it is important to keep in mind that the way we keep pigs in Sweden is very different from the way pigs are kept in Uganda. In Sweden, pig-farms usually house hundreds or thousands of pigs, in intense farming systems with carefully controlled breeding, feeding and medical treatment, and the use of antibiotics is strictly regulated. Pigs are often kept indoors, and have little or no contact with other animals, and people visiting usually change protective clothing before entering the stable. Many farmers are included in health-management programs to prevent diseases and to maximize animal welfare and profit. In Uganda, most farms have only a few pigs that are kept outdoors (Figure 5). Antibiotics and other drugs are accessible over the counter, and the veterinary services are expensive and not always easily accessible. There are many factors that differ in the management of pigs in Uganda and Sweden, which have an effect on health and antibiotic resistance.

Moreover, 88% of the isolates were resistant to sulfamethoxazole, although only one farm stated that they used this kind of antibiotics. The resistance to trimethoprim was less frequent, which make the results of the high percentage of sulfamethoxazole-resistance obscure since it is often combined with trimethoprim. However, oral treatment with sulfamethoxazole is used but since this is not an injection, the farmer might not consider it an antibiotic drug an does therefore not report it as such (E. Gertzell/M. Jacobson *pers. comm)*. This could explain the high percentage of sulfamethoxazole-resistance. During the interview we did not ask specifically about oral treatments with sulfa and therefore, the potential occurrence of such might have been overlooked. However, the author also had less experience in the reading of the micro-dilution plates, which also may have affected the results. It is sometimes hard to interpret the growth in the wells, especially regarding the reading of sulfamethoxazole where 20% of growth in the wells is supposed to be interpreted as negative. This was not noted until after all the readings were performed, thus these results should be interpreted with caution.

In the laboratory at Makerere University, a few possible sources of error occurred. Primarily, the pipettes were potentially not calibrated properly, in particular the multi-pipette that was used to fill the wells in the micro-dilution plates, was suspected to be incorrect. Also, the pipette tips were reused and autoclaved, a routine that may have altered their confirmation, possibly making the volume unspecific. Additionally, as stated above, the author had less experience in the reading of the micro-dilution plates, which may have affected the results. Being a low-income country, the facilities had a lower standard as compared to Sweden, and the temperature in the incubator as well as in the refrigerator varied and the laboratory hygiene was poorer, thereby increasing the risk of contamination. However, none of these factors seemed to have an impact on the study, basically because *E. coli* is a resilient bacterium. Nevertheless, there are challenges associated with performing a study in another country. Despite having an interpreter, not all the questions could be answered. It is hard to estimate what information is perceived by the interviewee the way it was intended and what gets lost in translation. The fact that we had two different interpreters alternating between the different field days contributes to the uncertainty. Sometimes, one also got the feeling that the interviewees did not really understand the question but answered anyway, for instance, imaginably the concept of antibiotics was difficult to grasp.

Most importantly, the increasing resistance against antibiotics worldwide is still a serious issue. As Jayarao *et al.* (2019) discusses in their report, the use of antibiotics can result in healthier and more productive animals with a lower rate of diseases, and it is a way to produce nutritious food at a low price. But at what cost? Since antibiotic resistance spreads through the food chain, the drugs we give to our food-producing animals will have an impact on human healthcare. We need to take action to save the antibiotics we have. There are other ways to keep healthy, productive animals without excessive use of antibiotics where the management and use of vaccines are two key attributes.

Standardized methods for testing of antibiotic resistance have been used in this study. For example, the method used in the Swedish surveillance program (Swedres-Swarm, 2018) is very similar to the one used in the present study, an exception however being that MacConkey-agar is used in the surveillance program. MacConkey-agar inhibits the growth of Gram-positive bacteria. However, it was not used in this study due to the limited space during the transport to Uganda and for economical reasons. *E. coli* is very easy to cultivate, so the estimation was that *E. coli* would be possible to culture and confirm nevertheless. *E. coli* was correspondingly found in all sampled but one.

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Figure 5. *A sow and her piglets resting in the shade (author, 2019).*

POPULÄRVETENSKAPLIG SAMMANFATTNING

Antibiotika är, för såväl människa som djur livsviktiga läkemedel som används för att bota infektioner orsakade av bakterier. Bakterier kan emellertid antingen ha en naturlig eller en så kallad förvärvad resistens mot antibiotika, vilket gör att de inte längre är känsliga mot antibiotika och överlever en behandling. Detta kan leda till längre sjukdoms- och behandlingstider, samt till att det kan krävas en annan behandling än den som tidigare varit effektiv. I slutänden kan det leda till att vi inte längre har några effektiva antibiotika mot sjukdomar som vi tidigare har kunnat behandla, till exempel lunginflammation och blodförgiftning. Dessutom, att delar av dagens sjukvård, så som avancerad kirurgi och neonatalvård inte längre är möjlig i samma utsträckning. Antibiotikaresistens är enligt Världshälsoorganisationen (WHO) ett av vår tids mest allvarliga, globala problem.

Naturlig resistens är något som förekommer ursprungligen hos vissa bakterier, förvärvad resistens däremot är något som bakterien erhåller, antingen genom mutation eller från andra bakterier, vilka kan dela med sig av gener till varandra. Den förvärvade mutationen drivs av naturligt urval, det vill säga att de bakterier som är resistenta överlever en antibiotikabehandling och förökar sig, medan de som inte är resistenta dör. I förlängningen drivs alltså resistensutvecklingen av antibiotikabehandling, vilket innebär att det är viktigt att använda antibiotika med eftertanke. Vissa typer av resistens hos bakterierna försvinner dock när det inte längre finns anledning för bakterierna att bära på den, det vill säga när antibiotika inte längre används.

I Sverige och andra delar av Europa, är antibiotikaanvändningen till människor och djur reglerad och övervakad. Svenska veterinärförbundet har riktlinjer för hur antibiotika ska användas till djur, och det är till exempel inte tillåtet att använda vissa typer av antibiotika som är reserverad för humant bruk. Antibiotika används när det finns en bra anledning till det, till exempel att man vet att det föreligger en bakteriell infektion. Användning av antibiotika i tillväxtfrämjande syfte förekom tidigare i Sverige, men förbjöds 1986. Det förekommer däremot fortfarande i stor utsträckning runt om i världen idag.

Ett sätt att övervaka resistensläget för antibiotika i en population, eller i ett land, är att undersöka resistensläget hos så kallade indikatorbakterier. Det görs bland annat i det svenska övervakningsprogrammet Swedres-Swarm, som övervakar antibiotikaanvändningen inom human- och veterinärvård. Genom att undersöka resistens hos indikatorbakterier kan man få en uppfattning om det övergripande resistensläget i en population. En bakterie som ofta används i studier och som rutinmässigt provtags på slaktsvin på svenska slakterier är *Escherichia coli,* en Gram-negativ bakterie som finns i tarmfloran hos alla varmblodiga djur. Den är vanligtvis ofarlig, men är en så kallad opportunist och kan orsaka sjukdom hos både djur och människor, såsom ödemsjuka och avvänjningsdiarré hos smågrisar och matförgiftning och urinvägsinfektion hos människa. *E. coli* kan dessutom både plocka upp och överföra resistensgener till andra bakterier, potentiellt även till sjukdomsframkallande bakterier, så kallade patogena bakterier, vilket gör att de kan fungera som reservoarer för resistensgener.

Den här studien är ett examensarbete inom Veterinärprogrammet avseende *E. coli* som en indikatorbakterie för resistens. Prover har tagits på grisar från små gårdar utanför Lira i norra Uganda. Totalt besöktes tjugo gårdar varav nitton hade grisar vid besöket. Vid besöket intervjuades en person på varje gård angående deras antibiotikaanvändning och prover togs från ändtarmen på grisarna. Besättningarna bestod av en till hundrafemtio grisar och en till tio grisar per gård provtogs. Proverna togs med provtagningspinnar som först odlades på blod- och CLED-agar i fältlaboratoriet i Lira. Med hjälp av bakteriernas utseende på agarplattorna samt oxidas-, kaliumhydroxid- och spot indol-tester kunde *E. coli* utskiljas på plattorna och bakterier sparades på nya provtagningspinnar. Bakterierna odlades sedan på nytt i laboratoriet vid Makerere Universitetet i Kampala. Därefter utfördes en resistensundersökning, där de enskilda bakterierna blandades upp i varsin buljong och distribuerades i mikro-titerplattor med olika typer av antibiotika i stigande koncentrationer. Samtidigt gjordes kontroll av att alla bakterier var i renkultur samt täthetskontroll av buljongen. Totalt togs 53 prov och *E. coli* isolerades i 52 av dem. De typer av antibiotika där mest resistens påvisades var mot sulfamethoxazole (88 %), tetracyklin (54 %) och trimetoprim (17 %). Tio av proverna var resistenta mot tre eller fler antibiotikaklasser, vilket innebär att de räknas som multiresistenta. Ett samband kunde påvisas mellan de gårdar från vilka resistenta bakterier isolerades och de gårdar som behandlat med antibiotika.

I den svenska övervakningsrapporten från 2018 angavs resistensen mot tetracyklin 9 %. Det är många faktorer som orsakar antibiotikaresistens i en population, och det är många faktorer i grishållningen som skiljer sig mellan länderna. I Sverige är grisproduktionen intensivare, gårdarna har ofta flera hundra grisar som är noggrant utfodrade, framavlade och det råder en strikt antibiotikapolicy, till skillnad från i Uganda där en gård oftast bara har några få grisar och djurhållningen är enklare, grisarna går utomhus, ofta i boxar eller bundna vid träd. Det går att köpa antibiotika på apotek utan att ha recept och veterinärvården är ofta dyr och inte lika lättillgänglig som i Sverige.

Det är många faktorer som kan påverka resultatet vid utförandet av en studie i ett låginkomst-land som kan påverka resultatet. Till exempel var kvalitetskontrollerna på laboratoriet på en lägre nivå jämfört med i Sverige och inkubatorn och kylskåpets temperatur varierade, något som kan påverka odlingen av bakterierna. Därtill var författaren oerfaren avseende att läsa av mikrotiterplattorna, något som också kan ha påverkat resultaten. Detta gäller framför allt resultaten för sulfamethoxazole, där en viss växt i brunnarna ända skall tolkas som ett negativt resultat. Det uppmärksammades inte förrän alla avläsningarna var genomförda, varför de resultaten ska tolkas med särskild varsamhet. Vad gäller enkätsvaren så är det alltid utmanande att arbeta med tolk, då det är svårt att avgöra vilken information som når fram och vad som misstolkas.

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APPENDIX 1

Questions

1. Have the pigs received any medical treatment with antibiotics during the last year?
   YES NO

If yes:

2. How often does the pigs receive treatment?
   1/WEEK 1/MONTH MORE SELDOM NEVER

3. Why did you treat with antibiotics?
   SICK ROUTINE TREATMENT OTHER

   a. If SICK:
      i. What symptoms did you treat?

   b. If ROUTINE TREATMENT:
      i. Why do you treat the pigs?

      ii. How often?

4. Did you or a veterinarian treat the pigs?
   YOU VETERINARIAN

   a. If YOU:
      i. Based on what grounds?
         SYMPTOMS TRADITION/HABIT OTHER

      ii. How did you choose what type of antibiotics to treat with?
         AVAILABLE TRADITION/HABIT OTHER

5. What dosage did they receive per pig and for how long?

6. Where do you usually buy antibiotics? Do you have anything at home, and can we have a look at it?

Farm:

Who was interviewed:

Number of pigs:

Status/impression of the farm:

Hygiene/observations:

Pig/sample	EC/SA	Healthy looking, signs?	Gender	Age	Treated with ab during last 12 months?

Number of SA-samples:
Number of EC-samples:

Other: