Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science

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Förekomst av antibiotikaresistenta *Staphylococcus aureus* i små grisbesättningar i Lira, Uganda

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SUMMARY

According to the United Nations (World Health Organization in particular) and the European Union, antibiotic resistance has become an enormous public health issue. Both veterinary and human medicine, and thus animal and human welfare, are at stake. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known variant of the common bacterium *S. aureus* that has acquired resistance to methicillin. The transferrable mutation in the bacterium makes it antibiotic resistant, which yearly leads to great impact in healthcare, and available treatments are limited. In Europe, several studies on the occurrence of MRSA in pigs are performed, but in Africa the studies are very few and in Uganda almost non-existent. In this study the occurrence of MRSA, alongside other *Staphylococcus* spp., in smallholder pig farms were studied. The samples were collected within the district of Lira, located in the northern region of Uganda.

The study included 51 samples from pigs: swabs taken from the snout, the skin behind the ears and the perineum from weaned pigs in nineteen different farms. The samples were analyzed by two pre-enrichment broths and on selective agar for MRSA, in parallel to culturing with one pre-enrichment broth followed by cultivation on bovine blood agar. In total, four isolates of *S. aureus* were obtained, of which one via the selective medium. The latter *S. aureus* was identified as MRSA through PCR, with demonstration of the genes for nuc, PVL and *mec*A. Another fifteen *Staphylococcus* spp. were found, of which four were resistant to at least three different classes of antibiotics, and thus can be regarded as multi-drug resistant.

CONTENT

ABBREVATIONS

- 1-S method – One enrichment step method
- 2-S method – Two enrichment step method
- CA-MRSA – Community-associated MRSA
- CAMHB – Cation adjusted Mueller Hinton broth
- CB – Clinical breakpoint
- CC – Clonal complex
- CGIAR – Consortium of International Agricultural Research Centers
- CNS – Coagulase negative *staphylococci*
- EC – European Commission
- ECDC - European Centre for Disease Prevention and Control
- EFSA – European Food Safety Authority
- EUCAST – European Union Committee on Antimicrobial Susceptibility Testing
- EURL-AR – European Union Reference Laboratory for Antimicrobial Resistance
- FAO – Food and Agricultural Organization
- FAOSTAT – Food and Agricultural Organization Statistics Division
- FDA – U.S. Food and Drug Administration
- HA-MRSA – Hospital-associated MRSA
- IFAD – International Fund for Agricultural Development
- ILRI – International Livestock Research Institute
- LA-MRSA – Livestock-associated MRSA
- MAAIF – Ministry of Agriculture, Animal Industry and Fisheries (Uganda)
- MALDI-TOF MS – Matrix-assisted Laser Desorption/Ionization – Time-of-flight Mass Spectrometry
- *mec* – A specific gene coding for methicillin resistance
- MHB – Mueller Hinton broth
- MLST – Multilocus Sequence Typing
- MRSA – Methicillin-resistant *Staphylococcus aureus*
- *nuc* – Nuclease, a specific gene
- OIE – World Organization for Animal Health
- PCR – Polymerase Chain Reaction
- PFGE – Pulsed-field Gel Electrophoresis
- PVL – Panton-Valentine Leucocidin (leucotoxin)
- SLU - Swedish University of Agricultural Sciences
- ST – Sequence type
- SVA – National Veterinary Institute (Sweden)
- TSB – Tryptic soy broth
- UBOS – Ugandan Bureau of Statistics
- UNAS – The Uganda National Academy of Sciences
- WHO – World Health Organization

INTRODUCTION

Uganda is a small country, located on the equator in eastern Africa. Its population is growing fast, and to date estimated to be approximately 44 million people, of which 41% are malnourished (FAOSTAT, 2019). Seventy-five percent of Uganda's population lives in rural areas and agriculture is by far the most important source of livelihood (UBOS, 2010). Chicken and goats are the most commonly held animals, followed by cattle, sheep and pigs. Nearly one-fifth of all households in Uganda keep pigs, and in 2017 the number of pigs were estimated to be 4.1 million (UBOS, 2018). Pigs are growing in popularity, since their reproduction and growth rate are high, and they are easy to keep and to sell. Pork consumption is increasing and during 2017, just over 24 000 tonnes of pork meat was produced. Beyond the value of the meat as food, a lot of people keep pigs as savings; they are easy to sell and can serve as financial resources in times of need. However, there are a lot of diseases and parasites that may affect the pig herds, and knowledge regarding correct feeding and management are often lacking, as is appropriate veterinary service (Dione *et al.*, 2014). Nearly 50% of the pig population is found in the central region of Uganda, while Lira, located in the northern territory, belongs to the areas with the least number of pigs owned (UBOS, 2008).

Antimicrobial resistance is today known as a major challenge in animal and public health worldwide. Among others, the United Nations (UN) and the European Union (EU) are concerned about this development and are taking actions to prevent its spread, through information and education. Although knowledge on antibiotic resistance in industrial countries is usually high, knowledge is generally low within low income countries, both at public and governmental level. In the World Health Organization (WHO) African region, only one out of 57 countries has a national plan for handling the future challenges of antibiotic resistance (WHO, 2017).

There are only a few studies conducted on *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in Uganda, and most studies concern human healthcare. In a study in South Africa, the herd prevalence of MRSA in pigs was found to be 12% (Van Lochem *et al.*, 2018) and in one study conducted in Senegal, the prevalence of MRSA in pigs was found to be 1.3% (Fall *et al.*, 2012), indicating a low to intermediate prevalence of MRSA in pigs in Africa.

The study was conducted in Uganda during September and October 2019, alongside another study investigating the occurrence of antibiotic resistance in *Escherichia coli* in pigs.

The aim of this study was to investigate the occurrence of antibiotic resistance in *Staphylococcus aureus* and in other *Staphylococcus* spp., and to compare the findings to owners' knowledge on the antibiotic usage in their pigs.

LITERATURE REVIEW

Pig husbandry in Uganda

Pigs in Uganda are gaining in popularity since they are omnivores, and therefore considered easier to feed than other livestock such as goats and cattle, easy to breed with their short reproductive interval, and are considered to be a fast growing species (ILRI, 2011; Atherstone *et al.*, 2018). In Uganda, holding pigs for selling and meat production is increasing as compared to animals such as goats and cattle, which have been more common historically (UBOS, 2008). Crossbred pigs are the most common, consisting of several different breeds, for example local indigenous breeds, landrace and large white (Muhangazi *et al.*, 2012).

From 1991 to 2008, the pig population increased from 0.67 million to 3.2 million in Uganda (UBOS, 2008). According to the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and the Ugandan Bureau of Statistics (UBOS) the number of pigs in 2017 were estimated to just above 4.1 million (UBOS, 2017a). With over 1.1 million pig owners, almost a fifth of Ugandan households, the majority keep a few pigs, and large piggeries are uncommon (UBOS, 2008). In a study in which questionnaires were handed out to pig farmers in central Uganda, 95% stated that they kept pigs for the possible income and only 5% for consumption of the meat themselves (Muhangazi *et al.*, 2012).

Despite this big and fast growth in pig holding, the sector claims to be overlooked by the government (Ouma *et al.*, 2015). However, the European Commission (EC) and International Fund for Agricultural Development (IFAD) in collaboration with the Consortium of International Agricultural Research Centers (CGIAR), has funded a research programme, the 'Smallholder Pig Value Chain Development" project. The aim of the project is to improve the livelihoods of small-scale pig producers in Uganda by improving their income, weighing in the challenges of climate change, and the project is being implemented with the help of the International Livestock Research Institute (ILRI; ILRI, 2011).

Pig management

There are three dominating types of housing for pigs in Uganda; tethering, housed and free-range/scavenging (Dione *et al.*, 2014). In rural areas, where many farmers are smallholders, tethering the pigs to a tree or similar is most common (See figure 1). In urban areas, housing is the most common type. The floor can for example consist of dirt, cement or concrete. This system can be expensive, depending on the materials used, and is more common in farms with at least five pigs. Free-ranging pigs are more common in rural than urban areas, but still quite uncommon. Mostly, piglets are left to scavenge for forage, since they pose little threat to the crops (Dione *et al.*, 2014). Feeding the pigs poses a challenge, due to several reasons: lack of knowledge on proper feeding, seasonal changes in availability and quality of feed, and the fact that many Ugandans are very poor (Dione *et al.*, 2014; FAOSTAT, 2019). The most common feeds given are leftover food from households or restaurants, forages and crop residues such as sweet potato vines or banana peels, cassava, potatoes or maize bran (Muhangazi *et al.*, 2012). Most often, water is offered twice a day, but some farmers only offer water once a day. During water scarcity some farmers don't offer water at all. The water source varies between tap water,

which is most common in urban areas, boreholes, rainwater, wells, springs and wastewater, whereas rainwater is the most common source in rural areas (Ouma *et al.*, 2015).

The most common way to extend the herd is farrowing, and by acquiring piglets as a gift or payment (Dione *et al.*, 2014). In rural areas, the practice of a village boar is common. The owner then allows other farmers' sows to be mated by the boar, and payment is often a piglet.

Several other different husbandry practices are also carried out by experienced farmers or village veterinarians, or paraprofessionals (Dione *et al.*, 2014). Practices such as castration, iron injections, feeding vitamin and iron supplements, deworming or ascaricides treatments against external parasite and teeth removal, occurs.

Figure 1. *Two tethered, sleeping pigs.*

Common diseases and treatment

Incidence of diseases in Ugandan pigs is estimated to be high. A study in 2012 revealed that 75% of the farmers experienced challenges and problems with diseases among their pigs (Muhanguzi *et al.*, 2012). Examples of diseases are African swine fever (ASF), Foot and mouth disease, worms and *Taenia solium* cysticercosis, diarrhoea, and cough. In addition, parasites such as mange, lice, mites, jiggers and ticks are also prevalent (Muhanguzi *et al.*, 2012; Dione *et al.*, 2014). Heat stress is a common problem, especially in rural areas where it more often leads to death than in urban areas (Dione *et al.*, 2014).

African swine fever is an endemic disease in Uganda, with outbreaks occurring all year round (Chenais *et al.* 2015). Recommended control and prevention for ASF is to quarantine and slaughter affected herds, disinfect the premises and restrict movement of pigs and meat in affected areas (Tatwangire, 2014). Humans are not recommended to eat affected meat, since this poses a risk for transmission of the disease to healthy pigs since they often are fed leftovers. However, it is reportedly not uncommon that farmers slaughter the pigs for meat and sell it cheap.

Several of the diseases in pigs in Uganda are preventable, but the lack of and high cost of veterinary services and drugs leads to poor implementation of preventative and control measures (Dione *et al.*, 2014). Furthermore, fake, expired and ineffective drugs are prevalent. Lack of knowledge on proper treatment among farmers and some practitioners is common, leading to high costs and unnecessary, and sometimes incorrect or inappropriate treatment.

Instead of consulting a veterinarian, farmers buy drugs from a drug shop and treat by themselves, or according to instructions given by the pharmacist or other staff (Muhanguzi *et al.*, 2012; Dione *et al.*, 2014). Some farmers treat sick pigs with local plants and herbs, and some farmers do not treat their animals at all (Muhanguzi *et al.*, 2012, Dione *et al.*, 2014).

The most common treatment carried out in livestock in Uganda is deworming (Amia *et al.*, 2019) and ivermectin or acaricides are the most common substances used (Dione *et al.*, 2014). To get rid of external parasites, scrubbing the pigs' skin with soap or covering them in mud is also common. The second most common treatment is antibiotics, and smallholder pig farmers use the latter extensively (Amia *et al.*, 2019). Antibiotics are easily accessed and administered, though legally, a veterinarian should prescribe and administer them (UNAS, 2015). Tetracycline is the most commonly used antibiotic (Amia *et al.*, 2019), but penicillins and flouroquinolones are also common (UNAS, 2015). Some farmers treat the pigs with antibiotics only when it's considered needed because of sickness or clinical signs, and some farmers administer antibiotics routinely, every week or month (Dione *et al.*, 2014). The belief that routine treatment with antibiotics prevents the pigs from getting sick is common.

Figure 2: *One farmers' medicine supply*.

Meat inspection and food safety

Food safety is a big challenge in Uganda, since adequate equipment to keep a safe cold-chain such as cooling trucks and refrigerators often are not available. One survey found that most pig traders clean their lorries after each use, but do not use any disinfectant (Atherstone *et al.*, 2018). Traders in the survey also stated that they were aware of the clinical signs indicating sickness in pigs, such as reddening or dropping of ears, straight tails and weakness or difficulties to stand. According to these traders, clinical signs are common, but rarely reported. In the same survey, traders stated that in 80% of the cases, they preferred to report any inaccuracies to a meat inspector rather than to a veterinarian. Action is rarely undertaken, but if it is, the pig is slaughtered, and the meat is sold on the market (Atherstone *et al.*, 2018).

In Kampala, slaughtered pigs for the urban market are regularly inspected at the Wambizi slaughterhouse, though the inspections are most often very superficial (Nsadha, 2013). In other

areas, food inspection occurs very irregularly, if at all, since slaughter takes place in many different places and at irregular times, and often in areas hard to access for veterinarians or meat inspectors. Illegal slaughter is also common in both rural and urban areas (Tatwangire, 2014). In a review on the prevalence of foodborne pathogens in animal-derived food from seven different countries in Africa, Uganda had the highest prevalence, at 50.8% (Paudyal *et al.*, 2017). *S. aureus* was the second most common bacteria found, next to *E. coli*.

Consumption of pork

Consumption of pork is increasing in Uganda and the number of slaughtered pigs in Kampala is estimated to be around 300 to 500 per day (Tatwangire, 2014). In 2017, official statistics counted a total of 24 000 tonnes of pork produced (UBOS, 2017b) and according to the International Livestock Research Institute (2011), 80 000 tons of pork is consumed annually. Pork consumption is mainly driven by cash availability and season, for example after harvesting coffee or at the beginning of the school terms, when pigs often are sold to pay for the school fees (Roesel *et al.*, 2019). The consumption also increases during holidays and festivals, such as Easter, Martyr's Day, Independence Day and Christmas.

Antimicrobials

The term antimicrobials refer to drugs or products that inhibit or kill microorganisms such as bacteria, virus, protozoa, fungi and parasites. This includes antibacterial, antimycobacterial, antiviral, antifungal and antiparasitic drugs (ECDC, 2019). Antimicrobial resistance emerges when microorganisms survive despite the presence of these drugs (EFSA-ECDC, 2017). Antibiotics are drugs that inhibits specifically bacteria and antibiotic resistance refers to resistance emerging in bacteria towards antibiotics, such as penicillin or tetracyclines (ECDC, 2019).

There are several different kinds of antibiotics, which are divided into different groups based on their chemical structure and their mechanisms of action (Vetbact, 2015). Some antibiotics work through inhibition of synthesis of the cell wall, which is the case in β-lactams such as penicillins and cephalosporins, and in glycopeptides, such as vancomycin. Since Gram-positive bacteria have a thick cell wall, these antibiotics works well, in contrast to the thinner and differently built-up cell wall of Gram-negative bacteria, where β-lactams and glycopeptides function poorly. Further, fluoroquinolones, for example enrofloxacin, and nitroimidazoles inhibit different steps in the nucleic acid synthesis. In Sweden, the use of fluoroquinolones in veterinary medicine is very restricted and regulated by legislation, and nitroimidazoles are not yet approved for animal treatment. Aminoglycosides, tetracyclines, macrolides and fusidic acid are examples of antibiotics that instead inhibit the protein synthesis of the cell. They can for example bind to and interfere with different parts of the ribosome or interfere with tRNA building amino acids. In folic acid antagonists, such as sulfonamids and trimethoprim, and furantoin, the mechanism of action is to inhibit different parts of the cell metabolism. These different mechanisms of action leaves numerous possibilities for the development of resistance (Vetbact, 2015).

Antibiotic resistance

In 1929, the discovery of penicillin was published by Alexander Fleming (Fleming, 1929), a discovery that revolutionized medical health care and treatment (WHO, 2015). In 1944, the first articles regarding antibiotic resistance were published (Kirby *et al.*, 1944; Spink & Vivino, 1944) and antibiotic resistance has since then continued to spread worldwide. Today, antibiotic resistance is a well-recognized global health problem and a huge challenge within medicine. The impact of antibiotic resistance does not only lead to decreased options for treatment and increasing prevalence of sickness or even death in humans, but also leads to consequences in veterinary medicine, animal welfare and food safety (Bengtsson & Greko, 2014). Furthermore, the impact of antibiotic resistance threatens to reduce productivity in both humans and animals, and lead to great impacts on national and international economies (WHO, 2015). In 2013, The World Economic Forum identified antibiotic resistance as a global risk and called it a challenge "*beyond the capacity of any organization or nation to manage or mitigate alone*" (Howell, 2013).

In May 2015, WHO released a Global action plan on microbial resistance in their Global Health Assembly. The purpose of this plan was to increase and improve the knowledge and education, and increase the evidence regarding antibiotic resistance, but also to reduce the incidence of infections, optimize antimicrobial medicines and develop a sustainable way of handling antibiotics. To achieve this, WHO, as a part of the United Nations, is collaborating both nationally and internationally, with the Food and Health Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE). WHO is not the only ones to react; in 2017 the European Union's Commission released "*EU One Health Action Plan against antimicrobial resistance*", and their three main objectives are to make the EU the best practice region, to boost research, development and innovation, and to shape the global agenda (EU Commission, 2015). One of the major goals is to heavily reduce the use of antibiotics, in humans as well as in animals.

Drivers of resistance

Antibiotic resistance is driven both by natural selection and genetic mutation. Misuse and overuse of antibiotic substances, as well as inadequate or non-existent surveillance and regulation, have increased the selection pressure, and are contributing drivers behind the rapid development of antibiotic resistance. In both presence as well as absence of selective pressure of antibiotics, bacteria may evolve intrinsic resistance, which is genetically stable and shared within the genus of the bacteria (McManus, 1997). Under selective pressure of antibiotics or even antimicrobials, acquired resistance can occur and this is of greater consequence. Acquired resistance leads to an alteration in the bacterial genome by spontaneous mutation and selection, or by the acquisition of extrinsic DNA (Tenover, 2006). This can lead to the bacteria acquiring traits that will destroy the antibiotic drug, stop it from reaching its target site, or even alter the binding site.

Acquired resistance can occur through horizonal evolution or horizontal gene transfer, which includes the mechanisms of conjugation, transformation and transduction (McManus, 1997). During conjugation, resistance genes are exchanged between bacteria with the help of plasmids, *i.e.* mobile DNA elements that can move directly between two bacteria. Conjugation through

plasmids is considered as one of the most common reasons for the spread of antibiotic resistance. Transduction is conducted between bacteria with the help of bacteriophages (bacterial viruses) and is an important mechanism of resistance in *S. aureus*, whose plasmids are not transmissible themselves. Transformation occurs when resistant bacteria release DNA material in the environment, and nearby bacteria take up the DNA and incorporate the genes in their own DNA (Tenover, 2006). This may occur after bacterial lysis caused by antibiotics such as monobactams or the third generation cephalosporins (McManus, 1997).

As earlier mentioned, the use of antibiotics is one of the main drivers behind the emergence of resistance. Antibiotic usage is high in many countries and is not only used as treatment in case of illness, but also used prophylactically and as growth promoters in livestock animals worldwide (Bengtsson & Greko, 2014). The prophylactic use of antibiotics and the use as growth promoters are controversial. In Sweden, the use of growth promoters was banned in 1986 (Jordbruksverket, 2019), and the EU followed the same line in 2006 (EC, 2005). The U.S. Food and Drug Administration recommended antibiotics as growth promotors to be phased out in the U.S. by 2013, however, it is still not banned (FDA, 2013). The use of antibiotics as growth promotors occurs in Uganda, but the extent is unknown and there are no laws prohibiting this use (UNAS, 2015). Furthermore, there are no specific programmes for surveillance or monitoring of any antimicrobial resistance trends in Uganda, and MAAIF does not monitor possible trends or publish any information on the subject. However, MAAIF is aware of the problem and the consequences of antibiotic resistance as a major public health concern (UNAS, 2015).

Staphylococcus aureus

S. aureus is Gram-positive commensal bacteria present on the skin and mucous membranes in both humans and animals, especially in the nares. Approximately 20-30% of all humans are asymptomatic carriers (Gordon & Lowy, 2008). In human medicine, *S. aureus* is one of the most common pathogens causing hospital-acquired infections (Archer, 1998). It is an opportunistic pathogen, and its potential to cause a wide variety of infections and infectious diseases depends on its ability to produce numerous virulence factors (Lowy, 1998, see Kong *et al.*, 2015 p. 2). For example, it can produce surface proteins that allow bacterial adherence, secrete extracellular toxins, produce enzymes leading to destruction of host cells and tissue, and it can grow and spread within the host cell. These virulence factors are the major reason for the success of *S. aureus* as a pathogen (Archer, 1998). Panton-Valentine leucocidin (PVL) is a well-known leucotoxin associated with *S. aureus* and MRSA (Gordon & Lowy, 2008). The PVL genes are carried on phages, and therefore has the ability to transfer between bacteria (Prévost *et al.*, 1995).

To identify different strains of *S. aureus*, the method of genotyping allelic variations through multilocus sequence typing (MLST) is often used (Maiden *et al.*, 1998). MLST tracks variations that accumulate over long periods and the method is highly reproducible. The *S. aureus* MLST method sequences internal fragments from 7 housekeeping loci (Feil *et al.*, 2003), and this method provides an allelic profile for each tested isolate, often referred to as "sequence type" (ST; Maiden *et al.*, 1998). MLST can potentially identify over one billion different STs and therefore similar STs are divided into groups of closely related STs, referred to as clonal

complexes (CC; Feil *et al*., 2003). According to the eBURST algorithm, a *S. aureus* CC is defined as groups where each isolate is identical to at least one other isolate in, at minimum, five of seven loci (eBURST, 2019; see Aires-de-Sousa, 2017 pp. 1).

Development of antibiotic resistance in S. aureus

There are several types of antibiotic resistance in *S. aureus*. Methicillin-resistant *S. aureus*, MRSA, is the most important type today, and it could have a major impact on the society. In addition, there are several other types of resistant *S. aureus*, for example phage-type 80/81 *S. aureus*, Vancomycin-intermediate *S. aureus* (VISA) and Vancomycin-resistant *S. aureus* (VRSA; Chambers & Deleo, 2009).

The two most important mechanisms behind antibiotic resistance in *S. aureus* are the production of β-lactamase or penicillinase, and the production of alternative penicillin-binding proteins (PBPs; McManus, 1997; Ogawara, 2019). Presence of β-lactamase or penicillinase leads to hydrolysis of the β-lactam ring in β-lactam antibiotics, which in turn leads to enzymatic inactivation and thus decreased antimicrobial activity (McManus, 1997). The gene behind this resistance mechanism, *blaZ*, is mostly located on plasmids (Weber & Goering, 1988) and may thus be transferred by sex pili (McManus, 1997). The second mechanism is the production of certain PBPs which have developed reduced affinity for all β-lactam antibiotics (Spratt, 1994). The mechanism behind methicillin resistance is the production of the earlier mentioned protein PBP2a, encoded by the *mecA* or *mecC*, a *mecA* homologue, genes, which is only found in MRSA and not in Methicillin-susceptible *S. aureus* (MSSA; Ito *et al*., 1999; Pichon *et al*., 2012). *mecA* is located on a mobile genetic element, the staphylococcal cassette chromosome *mec* (*SCCmec;* Pichon *et al.*, 2012). The structure of *SCCmec* varies between MRSA strains, but variation in the *mecA* gene is limited.

S. aureus was originally susceptible to all different types of antibiotics (Chambers & Deleo, 2009). During the mid-1940's, shortly after the introduction of penicillin on the market, the first penicillin-resistant *S. aureus* was discovered in hospitals. In only ten years, penicillin resistance became a prominent problem for the community and during the 1950's and 1960's, penicillin-resistant strains became pandemic (Roundtree & Freeman, 1956; see Chambers & Deleo, 2009 pp. 2). During the early 1960's, the first semisynthetic penicillin with decreased susceptibility to beta-lactamases, methicillin, was commercially available (Chambers & Deleo, 2009). The first published reports of a *S. aureus* strain with methicillin resistance came in 1961 (Barber, 1961). Emerging resistance to methicillin differed from the earlier resistance mechanisms as it showed no signs of drug inactivation and was showing resistance against both penicillins, cephalosporins and carbapenems (Chambers & Deleo, 2009). The *mecA* gene was discovered first 20 years later, in the 1980's. This second wave of antibiotic resistance mainly occurred in Europe, while the rest of the world were not as afflicted. After a decrease in resistance-related infections, outbreaks were again reported in the late 1970's and mid-1980's, especially in the U.S. (Crossley *et al.*, 1979; Peacock *et al.*, 1980, see Chambers & Deleo, 2009 p. 2). The strains reported during the 1970's and 1980's remain today, and though reported globally, *S. aureus* infections mainly afflict hospitals and healthcare institutions (Chambers & Deleo, 2009). After this third wave of antibiotic resistance, there was an increase in the use of vancomycin, an

antibiotic to which *S. aureus* still was susceptible, and due to the heavy selection pressure both VISA and VRSA emerged (Hiramatsu *et al.*, 1997; Heigel *et al.*, 2003).

Methicillin-resistant Staphylococcus aureus

MRSA is, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), defined as "*S. aureus isolates with an auxiliary penicillin-binding protein (PBP2a/PBP2c encoded by mecA or mecC genes) for which β-lactam agents have low affinity, except for the novel class of cephalosporins having anti-MRSA activity (ceftaroline and ceftobiprole)*" (EUCAST, 2017). MRSA is generally divided into three different categories: community-associated (CA-), healthcare-associated (HA-) and livestock-associated MRSA (LA-MRSA; EFSA-ECDC, 2018).

In Uganda, antibiotics have served as very important drugs in human healthcare, alongside for example anti-malarias (UNAS, 2015). In 1997, a study in major hospitals in Kampala showed a prevalence of 40 to 93% of MRSA among the *S. aureus* isolates (Mpairwe, 1997). Penicillin, ampicillin, and tetracycline had the highest percentage of resistance. Later, in 2011, in a study conducted in the burn unit, which however is considered a high risk population, at Mulago hospital in Kampala, 41 different *S. aureus* isolates were studied and all of them were found to be MRSA, and 63% of them where multi-drug resistant, *i.e.* resistant to three or more different classes of antibiotics (Kateete *et al.*, 2011). Few studies have been carried out, and the prevalence of MRSA has generally been high, however, methods for sampling and screening differ between studies and thus comparisons should be done with care.

MRSA in pigs

In animals, MRSA was first discovered in Belgium in 1972, in a case of bovine mastitis thought to be caused by human contamination (Devriese *et al.*, 1972, see Crombé *et al.*, 2013). Since then, MRSA has occasionally been found in various animal species and during the last 20 years, the reports have increased. A study conducted in 2005 presented a new type of MRSA found in pigs, which brought forth a new category, the earlier mentioned LA-MRSA (Voss *et al.*, 2005). The same sequence type of MRSA were found in pig farmers and their families, indicating that both transmission from animal to human, as well as transmission between humans, was possible. EFSA refers to LA-MRSA as "*a zoonosis with a direct public health impact*" and emphasizes the risks for certain professional groups such as veterinarians and pig holders, whose more common contact with the animals can be seen as an occupational health hazard (EFSA-ECDC, 2009). LA-MRSA is also thought to be able to transfer from the environment and via aerosols (Gilbert *et al.*, 2012; Agersø *et al.*, 2014). LA-MRSA in pigs most often belongs to clonal complex 398 (CC398; Voss *et al.*, 2005, Kinross *et al.*, 2017). Clonal complex 398 has also been found to be present in other livestock animals, such as cattle, poultry and horses (Baptiste *et al.*, 2005; Van Loo *et al.*, 2007; Kinross *et al.*, 2017).

The presence of MRSA in pigs does not normally entail any problem for healthy pigs (Kluytmans, 2010). There should neither be a concern in consuming MRSA-infected pork meat, since CC398 very seldom express the toxin genes that normally lead to food poisoning in *S. aureus* (Köck *et al.*, 2009).

MRSA in pigs in Europe

In Europe, the prevalence of MRSA in pigs varies highly between countries, from low to very high, with a mean of 26.9% within approximately 25 countries throughout Europe (EFSA-ECDC, 2009). Breeding holdings include farms where pigs are bred, born and held for growth to slaughter, while a production holding refers to a farm where weaned piglets are bought and held until slaughter. The former example is more closed, and not as exposed to for example transmittable diseases.

For example, in the production pig holdings, Spain has a herd prevalence of MRSA of 51.2%, Belgium 35.9%, and Germany 41.3%, of which the absolute majority is proved to be CC398 (EFSA-ECDC, 2009). These are countries with large pig populations held in large herds and in these countries, a high percentage of LA-MRSA in the human population is also noted. Other studies have also shown high prevalences of MRSA in production pig herds, for example in Belgium where 87% (Verhegge *et al.*, 2011) and 94% (Crombé *et al*., 2012) have been found. In Denmark, the prevalence of LA-MRSA in conventional pig herds measures to 89%, and accounts for 34% of the MRSA-related infections in humans (DANMAP, 2018). The prevalence of MRSA in Swedish pigs in 2019 is unknown but expected to be very low, since MRSA has only been found at very few times, for example once in two pigs in 2014 (Swedres-Svarm, 2014), and studies conducted later has not found any MRSA at all (Unnerstad *et al*., 2017). The occurrence of MRSA in food-producing animals in Sweden requires an official notification according to the regulation of the Swedish Board on Agriculture (1 ch. 12-14 § SJVFS 2013:24).

MRSA in pigs in Uganda

In opposite to high income countries, the prevalence of MRSA in pigs in Uganda, and Africa, is poorly surveyed. One study conducted at 147 different farms in the Kabale district in western Uganda, found an occurrence of 29.4% of MRSA, confirmed with polymerase chain reaction (PCR) for *mecA* (Andrew *et al*., 2018). Very high levels of resistance were reported to several different common antibiotics; 99% of the isolates were resistant to trimethoprim/ sulpha-metoxazole; 89% to erythromycin; 70% to clindamycin; 70% to tetracycline and 49% were resistant to gentamicin. Compared to studies in other African countries, the occurrence of 29.4% is very high: for example a study on commercial pig herds in South Africa showed a herd prevalence of 12% (Van Lochem *et al.*, 20018) and in one study conducted in one herd only in Senegal, an animal prevalence of 1.3% was seen (Fall *et al.*, 2012). The overall few studies in Africa and the lack of harmonized and standardized methods makes the prevalence of MRSA in pigs in Uganda hard to predict.

Diagnostics

Sampling methods

There are multiple sampling methods used in studies conducted on the prevalence of MRSA in pigs. Common practice is to sample the nares, such as in studies conducted by Verhegge *et al*., 2011; Fall *et al*., 2012 and Normanno *et al.*, 2015. Further, swabs from the perineum, or both perineum and nares, can be used, as in studies conducted by Ivbule *et al*., 2017 and Larsen *et al*., 2017. Unnerstad *et al*., 2017, used sterile cloths to swab the skin behind the ears. In the

study conducted by Voss *et al.,* 2015, pigs were sampled either in the nares or the perineum. The sensitivity of nasal swabs and ear skin-swabs was, in one study, 78% and 90%, respectively (Agersø *et al*., 2013). This study also included sampling of the environment in the comparisons, and when for example samples were plated directly from air filters, the sensitivity was 78%, but when taking dust samples, the sensitivity decreased to 43%. Another study comparing sensitivities presented results of 91.4% for the skin behind the ears, 76.5% for perineum and 75.3% for the nares (Pletinckx *et al*., 2012).

Culturing methods

A commonly used method to detect MRSA consists of one pre-enrichment step and one selective enrichment step, followed by culturing the enriched sample on selective chromogenic agar plates. This method is referred to as the 2-S (two enrichment steps) method by the European Union Reference Laboratory for Antimicrobial Resistance. Until June 2018, this was the recommended method for detection of MRSA in food-producing animals and farm environment (EURL-AR, 2018). However, it is important to remember that the methods for isolation of MRSA are not harmonised globally, and thus comparison and interpretation of data from different studies should be made with caution.

A pre-enrichment step utilizing Tryptic soy broth (TSB) has been shown to have a higher sensitivity as compared to pre-enrichment steps utilizing phenol red mannitol broth (PHMB), both with added cefoxitin and aztreonam (Böcher *et al.*, 2008). Combined with chromogenic agar of different brands, pre-enrichment with TSB showed a sensitivity for MRSA of 100% in all brands, compared to a sensitivity of 73-75% for PHMB. However, the sensitivity without any pre-enrichment step was reported to 98-99% for MRSA, but no specificity was presented. Antibiotic concentration was also shown to affect the results, and the use of 3 mg/L cefoxitin allowed growth of methicillin susceptible *S. aureus*, whereas the combination of 4 mg/L cefoxitin and >20 mg/L aztreonam led to a loss of sensitivity. Notably, Brilliance™ MRSA 2 was not one of the evaluated chromogenic agars in the study referred to (Böcher *et al.*, 2008).

Chromogenic agars such as the *Brilliance*™ MRSA 2 agar has been developed to allow fast and correct screening of MRSA in hospitalised patients (Veenemans *et al.*, 2013). MRSA grows in distinct, blue colonies and the selective proprietary agar withholding an "antibiotic cocktail", inhibits most susceptible bacterial strains and yeasts (Oxoid, 2001; Verkade *et al.*, 2009). Other examples on chromogenic specific agars for MRSA is ChromID MRSA, MRSA-ID and MRSA Select (Böcher *et al.*, 2008). Broth-enrichment steps are essential for a satisfactory diagnosis (Oxoid, 2001; Veenemans *et al.*, 2013). Sensitivity after direct inoculation can be as low as 52% (MRSA-ID) or 65.7% (*Brilliance*™ MRSA 2), compared to 98% and 100%, respectively, after broth enrichment (Veenemans *et al.*, 2013). The specificity for Brilliance™ MRSA 2 agar is 99.1% with the pre-enrichment broth step, slightly higher than MRSA-ID's 98.8%. According to Luteijn *et al.* (2011), the sensitivity of the culture on chromogenic agar is higher after 48 hours than after 18-24 hours, but the specificity decreases, genetic typing is however often made, which makes the decreased specificity less important. Culturing on chromogenic agar is common, but the pre-enrichment steps differ between various studies (Luteijn *et al.*, 2011).

A study in Denmark and Norway reported that the 2-S method led to a high ratio of false negatives regarding MRSA in pigs (Larsen *et al.*, 2017). The different steps were evaluated and compared to a method referred to as the 1-S (one enrichment step) method, where the second step of enrichment broth, tryptic soy broth (TSB) with cefoxitin and aztreonam, was surpassed. This method detected MRSA in 82% of the Danish samples, compared to the 74% detected with the 2-S-method, and in 5.6% and 3.8%, respectively, in the Norwegian samples. Therefore, the 2-S method is no longer recommended according to the EURL-AR.

Genetic analysis and susceptibility testing

Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry is considered a fast and reliable identification method for *Staphylococcus* spp. (Tang *et al.*, 2019), and is one of the most commonly used instruments (Hou *et al.*, 2019). The method is considered as Gold standard for microbial identification (Hou *et al.*, 2019). In genotyping, MLST is a common method and used, for example, in studies carried out by Fall *et al.*, 2012; Verhegge *et al*., 2012 and Normanno *et al*., 2015. Genotyping through PFGE or *spa* typing, and *SCCmec* typing for *mecA*- or *mecC*-positive strains by PCR, are also used in several studies.

In the detection of antibiotic resistance, minimum inhibitory concentration (MIC) determination is recommended (EUCAST, 2017). The method of broth microdilution in microtiter panels presents an easy and fast method of deciding whether bacteria are susceptible or resistant, and an advantage is that the method is easily standardized and automized (Kadlec *et al.*, 2019). The panels are custom or commercially manufactured, consisting of wells with freeze-dried antibiotics of different type and concentrations, alongside control wells without antibiotics. A disadvantage with the commercially manufactured microtiter panels is that the type and concentration of antibiotics is static and cannot be changed. For determining resistance, one can also use the method of agar disk-diffusion, which is easy and cheaper than microdilution. The bacteria, or the inoculum, is spread evenly on an agar plate, incubated, and the inoculum then creates a gradient in the agar plate. The diameter of the gradient is measured and compared to zone diameter breakpoints, and the result is interpreted as susceptible, intermediate or resistant (Kadlec *et al.*, 2019). A disadvantage is that no exact MIC values can be obtained, only approximated.

MATERIAL AND METHODS

This study was performed as a part of a research project on pig herd health management, conducted by SLU in collaboration with PhD student Elin Gertzell, ILRI and Makerere University. Twenty different farms in the district around Lira city, Uganda, were visited for sampling and a short structured interview was conducted, see appendix I. All the farms visited had previously signed an informed consent. This study was approved by the Institutional Ethical Review Committee in College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda (ref SBLS/REC/18/005) and approved by the Uganda National Council of Science and Technology (ref A591).

Sample collection

The samples were collected from the snout, the skin behind the ears, and the perineum, by the use of a swab transported in Amies medium with charcoal (Copan Diagnostics Inc., Brescia, Italy), from weaned pigs, or as close in age as possible. See figure 3. For each pig, one swab was used for all sampling sites. The number of samples on each farm was decided based on the total number of pigs and distribution in pens (See table 2, p. 16). The animals sampled were randomly chosen and, if possible, from different pens. Samples taken from herds with 25 pigs or more where pooled with two or three swabs in each tube, due to limited financial and laboratory resources.

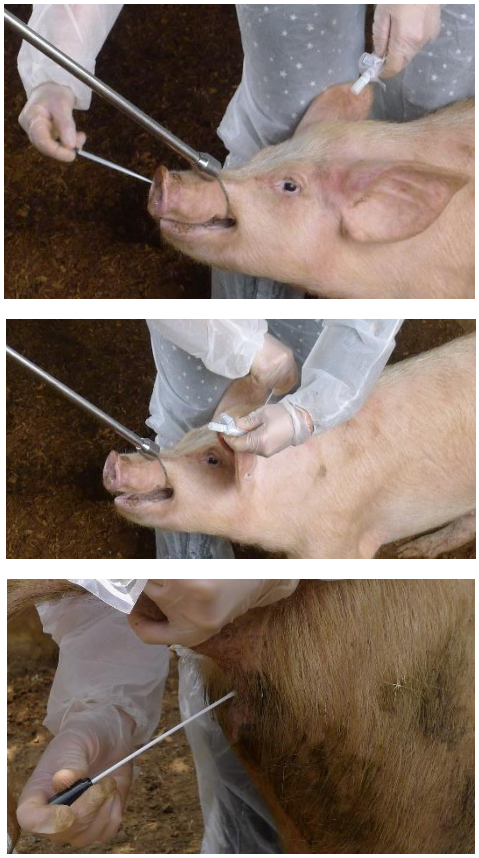

Figure 3. *Sampled sites demonstrated.*

Culturing and antibacterial resistance analysis

A total of 51 samples were included in the study and the culturing method used is known as the 2-S method, earlier recommended by the EURL-AR and employed by the European Food Safety Authority (EFSA-ECDC, 2017).

The samples originated from 87 pigs and 19 different farms. The samples were cultured either direct in Lira or stored in a refrigerator and cultured up to five days later, in Kampala. Due to equipment malfunction in Lira, several cultures were destroyed mid-culturing, but all original samples and tubes were however saved and re-cultured upon arrival to Kampala, up to eight days after the collection.

The swabs were enriched in 10 mL Mueller Hinton broth supplemented with 6.5% NaCl (SVA, Uppsala, Sweden) and incubated at 37°C for 24 h. Thereafter, the samples were homogenized by vortexing, 1 mL of the solution was added to 9 mL of Tryptic soy broth supplemented with 3,8 mg/L of cefoxitin and 72 mg/L of aztreonam (SVA, Uppsala, Sweden), and incubated at 37°C for 24 h. The broth was homogenized by vortexing and 20 µL was cultured on *Brilliance*™ Chromogenic Agar plates (Oxoid, Basingstoke, UK). The plates were incubated

at 37°C and growth was interpreted after 24 and 48 hours. Suspected colonies, light blue in colour, were streaked on 5% bovine blood agar plates (SVA, Uppsala, Sweden) and incubated at 37°C, and growth was interpreted after 24 and 48 hours. In plates containing bacteria in pure culture, the haemolysis was assessed, and several colonies in each isolate were tested with potassium hydroxide and catalase. Potassium hydroxide distinguishes Gram-positive bacteria from Gram-negative bacteria and the catalase test distinguishes *Staphylococcus* spp. from *Streptococcus* spp. and *Enterococcus* spp. Colonies with α- and/or β-haemolysis, that were catalase-positive and potassium hydroxide-negative were presumed to be *S. aureus* and stored in Amies transport medium with charcoal for further analysis of antibiotic resistance, conducted at the laboratory in Kampala.

Alongside conducting the 2-S method, all swabs in MHB were also cultured according to a 1-S method directly on 5% bovine blood agar (SVA, Uppsala, Sweden), and growth was assessed after incubation at 37°C for 24 hours. Suspected colonies were again sub-cultured on 5% bovine blood agar (SVA, Uppsala, Sweden) and incubated at 37°C for 24 hours. Suspected *Staphylococcus* spp. were chosen and evaluated based on appearance and haemolysis, and on the results from testing with potassium hydroxide and catalase. All catalase-positive and potassium hydroxide- negative samples with the correct appearance were considered presumptive *Staphylococcus* spp. Little consideration was put in the occurrence of haemolysis, since this can vary in-between different *S. aureus* strains.

Presumptive *S. aureus* isolates from the 2-S method and presumptive *Staphylococcus* spp. isolates from the 1-S method were analysed for antimicrobial resistance by determination of the minimum inhibitory concentration (MIC) using STAF/STREP panels (SVA, Uppsala, Sweden) and cation-adjusted Mueller Hinton broth (SVA, Uppsala, Sweden). The microdilution method was used according to the Clinical and Laboratory Standards Institute (CLSI; VET01/M100). The STAF/STREP panels include analysis of resistance for penicillin, cephalotin, oxacillin, enrofloxacin, fusidic acid, erythromycin, clindamycin, gentamicin, nitrofurantoin, tetracycline and trimethoprim-sulfamethoxazole. The samples were cultured on 5% bovine blood agar (SVA, Uppsala, Sweden) at 37°C for 24h. Five colonies, in total a full plastic loop of 1μL, from every cultured isolate was resuspended in 5 mL of cation-adjusted Mueller Hinton broth, CAMHB (SVA, Uppsala, Sweden), and incubated at 37°C for 3 hours. Thereafter, the culture was homogenised by vortexing, and 6 μL was added to 10 mL of CAMHB, vortexed, and 50 μL were inoculated in each well on STAF/STREP panels (SVA, Uppsala, Sweden). For purity control, the solution was also cultured on 5% bovine blood agar plates. Additionally, inoculum density control was conducted to secure the right concentration of bacteria, which is essential for the microdilution method. Ten μL from a random sample of the final 10-mL CAMHB-solutions were added to 10 mL of 0.9% sodium chloride, vortexed, and 10 μL was cultured on 5% bovine blood agar plates. The STAF/STREP panels, purity control and inoculum density control were all incubated at 37°C for 18 hours, before being interpreted. For interpretation of MIC values and classification as susceptible or resistant, clinical breakpoint values for *S. aureus* or catalase-negative *Staphylococci (S. cohnii, S. epidermidis, S. hyicus, S. sciuri* and *S. simulans*) issued from EUCAST (2019) were used. Except for oxacillin, the clinical breakpoint values were similar for all *Staphylococci* strains analysed. For species not listed in EUCAST, such as *S. chromogenes, S. lentus* and *S. petrasii*, values for *S. aureus* were used for interpret-

tation. For antibiotics not listed by EUCAST, *i.e.* cephalotin, enrofloxacin and fusidic acid, values from the 2018 Swedres-Svarm report were used (Swedres-Svarm, 2018).

The control strain *S. aureus* ATCC 29213 was included as quality control. The strain is methicillin-susceptible and recommended as a control strain for MRSA-testing (EUCAST, 2017).

Genetic analysis

The isolates were stored in a refrigerator in Amies medium with charcoal (Copan Diagnostics Inc., Brescia, Italy), before being shipped to Sweden where the isolates were confirmed as *Staphylococcus* spp. by MALDI-TOF mass spectrometry (Bruker, Bremen, Germany). Samples confirmed as *S. aureus* and resistant to oxacillin were analysed by Polymerase Chain Reaction (PCR) for the genes for *nuc* (a species specific marker), PVL (a gene coding for the MRSA-associated leucotoxin Panton-Valentine leucocidin) and *mecA* resp. *mecC* (genes coding for PBPs). In the PCR analysis samples were prepared according to the method used by Capurro *et al.*, (2009). One µL of bacteria was suspended in 50 µL lysostaphin (100 µg/mL in H_2O) and the solution was vortexed and incubated at 37°C for ten minutes. After incubation, 50 µL of proteinase K (20 mg/mL) and 150 µL 10 mM Tris-HCl (pH 7.25) were added, the solution was vortexed and incubated at 54°C for ten minutes. Thereafter, the solution was again vortexed and boiled for five minutes, cooled on ice for a minimum of ten minutes, and centrifuged for five minutes at 13 000 rpm. The supernatant containing DNA was stored at -20°C until the analysis by a modified PCR protocol according to Pichon *et al*., (2012).

RESULTS

Out of the 20 farms visited, one did not have any pigs at the occasion and was therefore not included in the study. Samples from six farms were pooled, with two or three samples in each pool. For pooling, the number of animals on the farm was on beforehand decided to be at least 25, however, two farms with only fifteen animals were also pooled (See table 1). An interpreter accompanied the group at all visits, but on some farms the owner or manager did not attend, the person interviewed could not always answer the questions, and therefore some answers are missing.

Table 1. *Number of pigs on each of 20 farms from the district of Lira at the time for interview and sampling, number of samples, number of pooled samples, and the age range of the pigs sampled*

Farm	Total number of pigs on farm	Total number of sampled pigs	Total number of samples after eventual pooling	Age range of sampled pigs
1	7	2	2	14-17 months
2	9	3	3	Unknown
3	18	3	3	7-13 months
4	3	1	1	10 months
5	126	21	7	6-8 months
6	4	1	1	8 months
7	2	1	1	5 months
8	16	4	4	6-7 months
9	15	6	3[1]	4-14 months
10	25	8	4	3-11 months
11	30	8	5	3 months, unknown
12	1	1	1	6 months
13	15	6	3	3-9 months
14	3	1	1	14 months
15	5	2	2	3-13 months
16	8	3	3	1.5-3 months
17	0	-	-	-
18	98	10	5	5 months
19	3	1	1	4.5 months
20	1	1	1	2.5 months
In total	389	87	51	1.5-17 months

[1] *Pooling was made after separation in different pens, hence two samples with two swabs in each, and two samples with one swab in each was taken.*

Owners knowledge on pigs' treatment

The questionnaire showed that 17 out of 19 farms (90%) had treated the pigs with antibiotics during the last year. The knowledge on the frequency and the duration of treatment was poor, but ten farms (53%) stated that they had treated their pigs twice or more. Three farms (16%) stated that their pigs had only been treated once, and four (21%) farms could not answer the question. Four farms (21%) stated that the pigs where treated "when needed" and four farms (21%) routinely treated their pig herds; either every third month (3 farms, 16%) or every month (1 farm, 5%).

The most common reasons to treat was sickness and clinical signs. Signs mentioned as reasons for treatment were wounds or injuries (n=6), skin problems (spots, red skin, lesions, loss of hair; n=5), diarrhea (n=3), weight loss (n=2), loss of appetite (n=2), low general state of health (n=2), ectoparasites such as lice or mites (n=2), fever (n=1), cough (n=1) or shivers (n=1). Ten farms (53%) claimed to treat only in case of sickness or clinical signs. In ten farms (53%), the person interviewed claimed that the treatments were always carried out by a veterinarian, who also brought the drugs: eight of these were farms who treated only in case of sickness or clinical signs. In three farms (16%), the treatments were essentially carried out by the owner or the manager, on the owner's decision, by drugs purchased at so called veterinary shops, were most often neither veterinarians nor pharmacists are employed. These farms also stated that they sometimes called a veterinarian, for example when experiencing complications after a castration, if the animals where sick and in one case, the farmer stated that they would call the veterinarian if their neighbor's pigs where sick, to receive treatment "just in case".

Only eleven farms (58%) knew which substances had been used. The most common antibiotic used was oxytetracycline, which had been used in ten farms (53%). Four farms had used a combination of penicillin and streptomycin. Tylosine and sulfamethazine had been used in one farm each (5%). One farm (5%) claimed that their pigs had never been treated with antibiotics, and another farm (5%) could not answer the question.

Occurrence of *Staphylococcus aureus* and *Staphylococcus* spp.

From the 51 samples collected, *S. aureus* was isolated from one sample (2%) by the 2-S method. When cultured in MHB and 5% bovine blood agar, *S. aureus* was isolated from three additional samples (6%). Totally, *S. aureus* was isolated from 4 out of 51 samples (8%). Through the culturing on bovine blood agar, another fifteen suspected *Staphylococcus* spp. were also found. Four suspected *S. aureus* and fifteen suspected *Staphylococcus* spp. strains were sent to Sweden, and confirmation with MALDI-TOF displayed nine different *Staphylococcus* spp., including four samples of *S. aureus*. The other *Staphylococcus* spp. demonstrated in one to five samples each belonged to: *S. simulans* (5), *S. cohnii* (2), *S. chromogenes* (2), *S. sciuri* (2), S. *epidermidis* (1), *S. hyicus* (1), *S. lentus* (1) and *S. petrasii* (1; Table 3).

Due to its resistance pattern, isolate number four (Table 3) was suspected to be MRSA and PCR analysis was conducted. Analysis established that the isolate was a MRSA as it was positive for *nuc*, PVL and *mecA* genes. The occurrence of MRSA in this study was therefore 2%. However, the study design did not in fact allow for individual prevalence estimates. Based on the results, one positive herd out of 19 sampled gives a herd prevalence of 5%.

Table 2. *Results of analyses by MALDI-TOF on 19 suspected strains of Staphylococcus* spp. *originating from pigs in the district of Lira in Uganda*

Isolate no	Farm	Pooled (number of swabs in each sample)	Result with MALDI-TOF
1	Farm 5	Yes (3)	*S. aureus*
2	Farm 8	No (1)	*S. aureus*
3	Farm 13	Yes (2)	*S. aureus*
4	Farm 15	No (1)	*S. aureus (MRSA)*
5	Farm 3	No (1)	*S. simulans*
6	Farm 9	Yes (2)	*S. simulans*
7	Farm 11	No (1)	*S. simulans*
8	Farm 11	No (1)	*S. simulans*
9	Farm 18	Yes (2)	*S. simulans*
10	Farm 5	Yes (3)	*S. cohnii*
11	Farm 5	Yes (3)	*S. cohnii*
12	Farm 6	No (1)	*S. chromogenes*
13	Farm 5	Yes (3)	*S. chromogenes*
14	Farm 9	No (1)	*S. sciurii*
15	Farm 20	No (1)	*S. sciurii*
16	Farm 14	No (1)	*S. epidermidis*
17	Farm 19	No (1)	*S. hyicus*
18	Farm 11	Yes (2)	*S. lentus*
19	Farm 12	No (1)	*S. petrasii*

Occurrence of antibiotic resistance

Of the four confirmed *S. aureus* isolates, all were resistant to penicillin. One isolate (no 4) had a resistance pattern strongly indicative of MRSA. One other *S. aureus* isolate showed resistance to erythromycin (>2 μg/mL; see Table 4).

Table 3. *Distribution of MIC values (μg/mL) among confirmed* S. aureus *isolates. Pc = penicillin, Ct = cephalothin, Ox = oxacillin, Fox = cephoxitin, Ef = enrofloxacin, Fu = fusidic acid, Em = erythromycin, Cl = clindamycin, Gm = gentamicin, Ni = nitrofurantoin, Tc = tetracycline, T/S = trimethoprim/sulphametoxazole, CB = clinical breakpoint values (EUCAST, 2019; Swedres-Svarm, 2018). Values marked in purple and red are interpreted as resistance*

Isolate	Pc	Ct	Ox	Fox	Ef	Fu	Em	Cl	Gm	Ni	Tc	T/S
CB	>0.125	>1	>2	>4	>0.5	>1	>2	>0.5	>1	>32	>2	>1/19
1	1	<1	<0.25	4	<0.25	<0.5	<0.5	<0.5	<1	<16	<0.25	0.25/4.75
2	>1	<1	<0.25	4	<0.25	<0.5	<0.5	<0.5	<1	<16	<0.25	0.5/9.5
3	>1	<1	0.5	4	<0.25	<0.5	>2	<0.5	<1	<16	<0.25	0.5/9.5
4	>1	2	>1	>8	<0.25	<0.5	>2	<0.5	<1	<16	<0.25	0.5/9.5

The resistance pattern of *Staphylococcus* spp. other than *S. aureus*, varied. The most common antibiotic resistance present was against tetracycline (seven isolates), penicillin (six isolates) and fusidic acid (five isolates). Four isolates each showed resistance to oxacillin and clindamycin. Three isolates were resistant to cephoxitin, two isolates against erythromycin and one each against gentamicin and trimethoprim/sulphametoxazole, respectively. No isolates were resistant to cephalotin, enrofloxacin or nitrofurantoin. Overall, the resistance varied among the different isolates, except for two isolates, 10 and 11, that both were resistant to penicillin, oxacillin, cephoxitin, fusidic acid, erythromycin and tetracycline. Isolate no. 10 was also resistant to clindamycin (Table 5). These two samples belonged to the same subspecies, *S. cohnii*, and originated from the same farm, but from different pens. The five different isolates of *S. simulans* were resistant to one type of antibiotic only, if any at all. Four isolates may be considered as multi-drug resistant.

Table 4. *Distribution of MIC values (µg/mL) among confirmed* Staphylococcus *spp. isolates. Clinical breakpoint values according to EUCAST or Swedres-Svarmpat 2018, for either S. aureus or coagulase-negative staphylococci. Pc = penicillin, Ct = cephalothin, Ox = oxacillin, Fox = cephoxitin, Ef = enrofloxacin, Fu = fusidic acid, Em = erythromycin, Cl = clindamycin, Gm = gentamicin, Ni = nitrofurantoin, Tc = tetracycline, T/S = trimethoprim/sulphametoxazole, CB = clinical breakpoint values (EUCAST, 2019; Swedres-Svarm, 2018). Values marked in purple and red are interpreted as resistance*

Isolate	Pc	Ct[1]	Ox	Fox	Ef[1]	Fu	Em	Cl	Gm	Ni[1]	Tc	T/S
CB	>0.125	>1	>0.25	>4	>0.5	>1	>2	>0.5	>1	>32	>2	>1/19
5	<0.03	<1	<0.25	2	<0.25	1	<0.5	<0.5	<1	<16	>4	<0.25/4.75
6	<0.03	<1	<0.25	2	<0.25	0.5	0.5	0.5	<1	<16	0.5	<0.25/4.75
7	<0.03	<1	<0.25	2	<0.25	<0.5	<0.5	<0.5	<1	<16	>4	<0.25/4.75
8	<0.03	<1	<0.25	4	<0.25	1	<0.5	<0.5	<1	<16	<0.25	<0.25/4.75
9	<0.03	<1	<0.25	2	<0.25	1	<0.5	1	<1	<16	0.5	0.5/9.5
10	0.5	<1	1	>8	0.5	>2	>2	1	<1	<16	>4	<0.25/4.75
11	0.25	<1	0.5	8	<0.25	>2	>2	<0.5	<1	<16	>4	<0.25/4.75
12[2]	<0.03	<1	<0.25	0.5	<0.25	<0.5	2	<0.5	2	<16	0.5	<0.25/4.75
13[2]	>1	<1	<0.25	1	<0.25	<0.5	1	<0.5	<1	<16	0.5	>4/76
14	0.06	<1	1	2	0.5	>2	<0.5	1	<1	<16	>4	<0.25/4.75
15	0.06	<1	1	2	<0.25	2	<0.5	<0.5	<1	<16	<0.25	<0.25/4.75
16	<0.03	<1	<0.25	2	<0.25	<0.5	<0.5	<0.5	<1	<16	>4	<0.25/4.75
17	>1	<1	<0.25	0.5	<0.25	<0.5	<0.5	<0.5	<1	<16	>4	1 / 19
18[2]	0.25	<1	1	2	0.5	2	<0.5	1	<1	<16	0.5	<0.25/4.75
19[2]	>1	<1	<0.25	2	<0.25	<0.5	<0.5	<0.5	<1	<16	0.5	<0.25/4.75

[1]*Source: Swedres-Swarm, 2018*
[2]*Values for S. aureus in EUCAST*

DISCUSSION

Occurrence of *Staphylococcus* spp. and antibiotic resistance

In this study on the occurrence of antibiotic-resistant *Staphylococcus aureus,* 19 different isolates of *Staphylococcus* spp. were isolated from 51 samples. Only two of the isolates weren't resistant to any antimicrobials tested.

Out of the 19 *Staphylococcus* spp., four were identified as *S. aureus* and one of them as MRSA. The isolate originated from a farm where the animals were routinely treated every third month and also when they were sick. All treatments were carried out by a veterinarian. The veterinarian usually brought the antibiotics, and the person interviewed stated that treatment was carried out with four different types of oxytetracyclines and tylosine. It is however unknown if they were used separately or combined and how the antibiotics were given. These routines are similar to some other farms in this study where no MRSA was found, but the overall high use of different antibiotics probably creates a higher selection pressure.

Of the other *Staphylococcus* spp., seven out of fifteen isolates were only resistant to one of the tested antimicrobials. Isolates of *S. simulans* appeared to be less prone to develop resistance, as compared to both strains of *S. cohnii* that showed resistance to several different antibiotics. The different isolates of *S. simulans* were mainly found on farms where pigs were treated routinely and had been treated several times during the last year, even though little resistance was found. The two isolates of *S. cohnii* originated from the same farm, where the owner most often treated the animals himself "when needed", depending on sickness and/or clinical signs, and with both oxytetracycline and penicillin-streptomycin. The farm was large (126 pigs) and, according to the manager, information regarding animals and treatments were written down, however, information regarding how often animals were actually treated, could not be retrieved at the time of the visit.

Multiresistant isolates were found in samples from the three largest farms (30, 98 and 126 animals), indicating that the number of pigs impact the prevalence of antibiotic resistance. However, on the third largest farm, two isolates of *S. simulans* with no or little resistance were also found, indicating that the type of *Staphylococcus* spp. is also important for the emerge of resistance. The single isolate of MRSA was found on a farm with only five pigs, where treatment were made irregularly by the manager himself. Correct treatment from educated personnel and compliance of the owners is probably the most important factor for preventing the emerge of antibiotic resistance.

In one study conducted on the occurrence of *Staphylococcus* spp. in fermented foods in west and central Africa, two isolates of *S. cohnii* and one isolate of *S. simulans* were found (Ouoba *et al.,* 2019). Expression of resistance genes were analyzed and both *S. cohnii* isolates were resistant to eleven out of nineteen different antimicrobials included in the study, making it the most resistant type out of all the *Staphylococcus* spp. found. In the *S. simulans* isolate, no expression of resistance genes was found. Another study conducted on swine meat in Italy, found five isolates of *S. simulans*, which all held resistance to at least two types of antibiotic classes (Simeoni *et al.,* 2008). Several articles often refer to *S. simulans* and *S. cohnii* as *coagulase-negative Staphylococci* among others, but when pointed out, *S. cohnii* seems to be

more prone to develop antibiotic resistance than *S. simulans*. In one study, both resistance to erythromycin and certain genes specific for antibiotic resistance in *S. cohnii* were found (Nobrega *et al.*, 2018).

Two other isolates were regarded as multiresistant: one *S. sciuri* and one *S. lentus*. These originated from different farms where little or no information about recent treatment could be collected. *S. sciuri* has been shown to carry the *mecA* gene coding for resistance against beta-lactams and genes coding for tetracycline resistance (Simeoni *et al.*, 2008; Ouoba *et al.*, 2019). The same studies also presented *S. lentus* with the gene coding for tetracycline resistance, however, the number of isolates of *S. lentus* were few (Simeoni *et al.*, 2008; Ouoba *et al.*, 2019).

There are few studies conducted on the occurrence of antibiotic-resistant *S. aureus* in humans in Uganda, and in pigs, they are even fever. One study conducted on nearly 600 Ugandan pigs showed a high prevalence, 29.4%, of MRSA (Andrew *et al.*, 2018). In the UNAS "Antibiotic Resistance in Uganda: Situation Analysis and Recommendations" from 2015, in a study conducted by Kalule *et al*. (2014), the occurrence of MRSA in pigs and vervet monkeys was claimed to be 64%, of which 73% were multi-drug resistant (Kalule *et al.*, 2014, see UNAS, 2015). These figures differ very much from the result in our study, and in the study by Andrew *et al.* (2018), ten times more animals were sampled as compared to our study. The method used is commonly used, but differs from the one used in this study, whereas instead of bovine blood agar or specific media for MRSA, mannitol salt agar was used for culturing after a pre-enrichment step, and for testing suspected isolates, catalase, tube coagulase and DNase test were used. Susceptibility was tested with a method of disk-diffusion, a reliable but less exact method for measuring MIC values. The study was carried out in Kabale, a district in the southwest of Uganda which has similar pig density and socioeconomic requisites as in the district of Lira. Both these papers (Kalule *et al.*, 2014; Andrew *et al.*, 2018) are however published in a non-indexed journal and not indexed by any search engines commonly used for scientific reports. The former paper, Kalule *et al*. (2014) could not be retrieved at all and information was retrieved from UNAS (2015), hence no evaluation of the method used can be made.

In Denmark, the prevalence of MRSA in conventional pig herds has been increasing over the years, from about 5% in 2008 to 65% in 2014 and then levelling out at 88% in 2016 and 89% in 2019 (DANMAP, 2019). Notably, testing free-ranging pig herds, both organic and conventional herds, resulted in a lower prevalence: 6% in 2015 with an increase to 20% in 2018. However, these results originated from smaller surveys that differ in sample selection and method, thus the results are hard to compare with the studies conducted on conventional pig herds. However, the sample size and the management structure such as access to open air and low animal density might make them more appropriate for comparisons with the results obtained in this study. Organically kept Danish pigs also receive less antibiotics than conventionally kept (DANMAP, 2019), The lower density of animals and less treatments with antibiotic might lead to a decreased selection pressure compared to conventionally kept pigs, which could be the reason for the overall lower prevalence of MRSA.

In Sweden, there is no regular surveillance of the occurrence of MRSA in pigs, but a few studies have been performed: in total only two positive samples, out of 191, were found in 2010 (Swedres-Svarm, 2011; Swedres-Svarm, 2014; Swedres-Svarm, 2018) and none in the latest

screenings 2011 and 2014, where 4734 and 3444 pigs respectively, were sampled (Unnerstad *et al.*, 2017). The few MRSA samples cannot be linked to a particular herd or farm. Up to 2018, the findings of MRSA in animals in Sweden is sparse, with a total number of positive samples of 112, whereas horses and dogs are the most commonly affected animals.

Even if the results in this study are similar to previous results in studies from Sweden, comparing the occurrence of MRSA findings in Uganda and Sweden is dubious. Several aspects, such as management, antibiotic treatment and knowledge, differ substantially. The farms visited mostly kept up to twenty pigs, while commercial piggeries as the ones in Sweden, often hold several thousand of pigs. Furthermore, several aspects of the management differ, such as how the pigs are housed, whether they have contact with other pigs or other animals or not, and basic hygiene routines. In Sweden, treatment with antibiotics is strictly regulated, whereas in Uganda, antibiotics are easily accessed and sometimes used for routine treatment. Moreover, treatment in Uganda is not always conducted by trained professionals, and even then, not always made according to recommended routines. In Swedish piggeries several different hygiene routines are implemented, with the aim to minimize the risk of transmission of various pathogens between the herds. In Uganda, pigs are kept outdoors, sometimes free-ranging, and uncontrolled selling and buying of pigs is common. Moreover, the use of a village boar is common, allowing pathogens and microorganisms to move in-between the herds. Biosecurity in pigs and the pig density in Uganda is very low as compared to the situation in Sweden. The density of animals has been discussed to have an impact on the spread of MRSA in pigs, and Alt *et al.* (2011) has shown that piggeries with over 500 individuals are more likely to carry MRSA.

Conducting a study abroad and adapting the method

Conducting a study in a low-income country brings on different difficulties, both big and small. Language can be a substantial barrier, and even though an interpreter was brought to every farm, the person interviewed could not always answer the question. There were two different interpreters involved, depending on the actual farm, both with some background in animal husbandry or veterinary medicine. It is hard to know whether the questions or answers got lost in translation or whether the person interviewed did not know or did not want to answer. One must also consider the fact that previously unknown foreign students were entering the premises when the samples were collected and the interview was given. This could lead to farmers either not wanting to talk, because of for example shyness, or wanting to give a positive appearance of their farm and animals, and therefore withholding important information.

Furthermore, we do not know the educational background of the farmers visited, which leads to another dimension of the lack of answers: if one does not know what an antibiotic is – how can you tell if you treated your pigs with it? Still, only two farmers (11%) claimed to not know whether the pigs received any treatments during the last year or not. All farms which stated that the pigs had received treatment could tell who performed them.

Sampling and pooling were planned from a schedule made beforehand. From the beginning, the study was planned to include approximately 60 samples, but since several farms had less pigs than expected, the final number of samples ended up to 51. Hence, trying to keep the

schedule, the sampling was made a little bit more spontaneously than was expected. Some farms had no weaned piglets, and thus the pigs closest in age were sampled. This also led to sampling of grown-up pigs in the absence of younger ones. Pooling was always made according to the distribution of pigs in pens. For comparison of the results and size of this study, one could compare two studies carried out in Senegal and South Africa, which handled samples from circa 450 pigs each (Fall *et al.*, 2012; Van Lochem *et al.*, 2018), and where a prevalence of 12% and 1.3%, respectively, was found. A broader selection and a larger sampling size might have increased the possibilities of detecting more MRSA in this study, but the prevalence in Van Lochem *et al.* (2018) is very similar.

Sampling was made from the snout, the skin behind the ears and the perineum with the same swab. Since assistance was provided to hold the pigs, there was no problem to avoid the swab from touching other surfaces. However, all sampling was made in pens, stables or outside the buildings, and thus some contamination from for example the environment cannot be excluded. However, this should also infer contamination of the pigs living in this environment.

In Lira, unused material and samples were stored in different refrigerators, at around 10°C and the refrigerator where the unused material was kept was not clean. The incubator used was not aimed for culture of microorganisms, instead the manufacturer focuses on storing reptile eggs. After six days (15th September) the incubator broke down and all agar plates inside were disposed of. These samples were re-cultured in Kampala, where we had access to a superior lab.

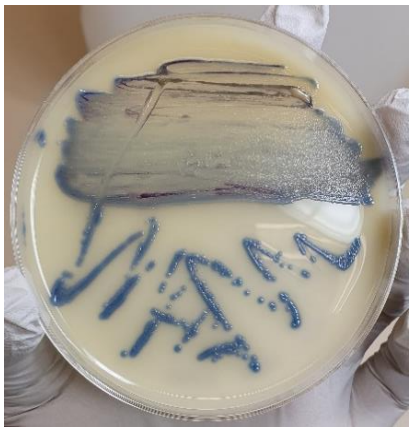

Figure 4. *Suspected MRSA colonies on chromogenic agar*.

The method used is specifically designed for determination of MRSA, which was the main objective of this study. The specific MRSA2 Brilliance™ agar plates allow MRSA strains to grow in a denim-blue color. The material was tested in Sweden beforehand, but well in Uganda few typical, light blue-colored colonies could be found. Colonies were ranging in color from very light blue or grey, to dark purple, which made the initial interpretation difficult (See figure 4 and 5). Suspected colonies were chosen as close as possible in color and when colonies were questionable, additional culture was made. A lot of other bacteria were growing on the specific media, in white, yellow and pink colors, but should be ignored according to the manufacturer, *e.g.* pink colonies indicate growth of *Bacillus licheniformis*. The two studies previously mentioned by Fall *et al.* (2012) and Van Lochem *et al.* (2018) both used specific chromagar plates (BBL CHROMagar™ Staph aureus and BBL CHROMagar™ MRSA; chromID™ MRSA). Van Lochem *et al.* (2018) cultured directly onto the specific agar, and a number of samples from different herds were cultured including a step of pre-enrichment broth (thioglycolate broth) as control. Fall *et al.* (2012) inoculated all samples in a pre-enrichment brain-heart infusion broth supplemented with colistin (4 mg/L), at 37° for 24 hours. The method used in this study differed slightly in type of broth, presence of antibiotics and in the trademark of the chromogenic agar, but execution is

still similar. Unfortunately, there is no harmonized method or Gold standard for analyzing of *S. aureus* or MRSA as of today, which makes comparisons between different studies difficult.

The culturing method used in this study including two steps of enrichment broth, has in a previous study proved to give a high number of false-negative results (Larsen *et al*., 2017). Another study was however not able to show any significant difference between using one or two different enrichment broth steps (Nemghaire *et al.*, 2014). Because of the study conducted by Larsen *et al.* (2017), and available lab resources, all samples were pre-enriched in Mueller-Hinton broth and thereafter directly cultured on 5% bovine blood agar (SVA, Sweden, Uppsala) for comparisons. The samples were stored in a refrigerator in MHB for eight up to fifteen days but this should not have had any major effect on the results. Profuse growth of several different bacteria, yeast and mold was found in several samples, for example suspected *Bacilli* and *Proteus* spp.

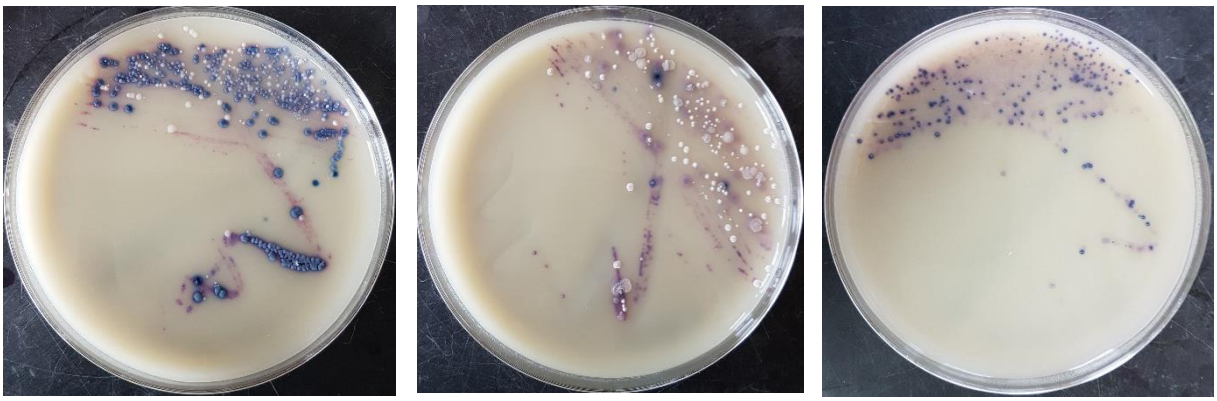

Figure 5. *Mixed growth on chromogenic agar. On these, no presumptive MRSA was found.*

In conclusion, a different culture method would probably not have yielded a different result and the 2% prevalence of MRSA in smallholder pig farms seems reasonable. One should consider the small number of pigs sampled, and the fact that herds were only sampled once and not repeatedly. There are some factors supporting our results such as the possible influence of a lower pig density, which has been shown to impact the prevalence. However, biosecurity and hygiene routines are rarely good, which could indeed have a negative impact. A few farmers testified to treating the animals routinely, and since antibiotic treatment is considered one of the strongest drives behind the spread of antibiotic resistance, this could lead to the low level of antibiotic resistance found.

No confidence interval was calculated as herds were not randomly selected and the sampling protocol did not allow for reliable statistics. However, the small size of the study and the sampling challenges should be taken into account when interpreting results.

Conclusion

Despite uncontrolled use of antibiotics and poor biosecurity, this study found a low prevalence of MRSA in Ugandan smallholder pig farms. Further studies are greatly needed before any conclusions of the prevalence of MRSA in smallholder pig farms in Uganda can be taken.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Syftet med det här examensarbetet var att undersöka förekomsten av en specifik typ av antibiotikaresistent bakterie hos grisar i ett område i Uganda. Antibiotikaresistenta bakterier, det vill säga bakterier som inte kan behandlas med antibiotika som t ex penicillin, blir till synes vanligare och vanligare överallt i världen. Anledningen till att man vill veta mer om detta, är att antibiotika är ett läkemedel som spelar stor roll inom både veterinär- och mänsklig vård. Tack vare alla de olika antibiotikatyper som finns idag, kan vi nuförtiden bota sjukdomar och medicinska tillstånd som förr i tiden var livshotande i högre grad, t ex lunginflammation och blodförgiftning. När bakterier utvecklar ett försvar mot antibiotika leder detta till många möjliga problem inom sjukvården.

Bakterien som letades efter var *Staphylococcus aureus*, vilken ibland kallas gula stafylokocker, vilket är en bakterie som förekommer normalt hos människor och djur. Hos friska individer utgör den oftast ingen risk, men den räknas ändå som en patogen bakterie, det vill säga en bakterie som kan framkalla sjukdomar. Den trivs extra bra i och runt munnen samt näsan hos människor. Hos grisar hittar man den oftast i trynet, på skinnet bakom öronen eller runt anus. Om människan eller djuret som bär på den av någon anledning får nedsatt immunförsvar, till exempel vid sjukdom eller skada, så kan bakterien föröka sig och orsaka infektioner som är svåra att behandla. *S. aureus* är känd för sin stora förmåga att producera flera olika typer av gifter, vilket kan leda till olika typer av sjukdomstillstånd. Till exempel kan bakterien ge upphov till variga sårinfektioner, bölder, matförgiftning och urinvägsinfektioner. *S. aureus* är också en vanlig anledning till toxic shock syndrome, TSS, den så kallade tampongsjukan hos människa.

Beroende på vilken typ av antibiotika man använder, så kan den verka på olika sätt: antingen genom att hindra tillväxt av bakterien eller genom att döda bakterien. En av de vanligaste typerna av antibiotika är penicillin, vilken verkar genom att förstöra bakteriens cellvägg, så att bakterien dör. Andra antibiotikaklasser verkar på genetisk nivå och förhindrar att bakterien förökar sig. Antibiotikaresistens innebär att bakterier utvecklar ett försvar mot de läkemedel som ingår i gruppen antibiotikum. Detta är ett stort problem över hela världen, eftersom antibiotika är en av få läkemedelstyper som kan behandla och bota sjukdomar som orsakas av bakterier. Genom att bakterierna utvecklas och muterar kan, som tidigare nämnts, de olika mekanismerna kringgås.

Vad gäller *S. aureus* finns det flera olika typer av resistens som kan utvecklas, men den som oftast nämns är MRSA: methicillinresistent *Stafylococcus aureus*. Methicillin är en typ av antibiotika inom gruppen penicilliner, och methicillinresistens hos *S. aureus* isolerade från människa upptäcktes redan 1961. På djur påvisades MRSA första gången 1972, på en ko med juverinflammation. Man misstänkte då att djuret hade smittats av en människa. Sedan dess har flertalet rapporter om MRSA hos djur och människor publicerats, och under de senaste 20 åren har rapporterna ökat i omfattning. I en studie gjord på grisar i Danmark 2005 påvisades en ny typ av MRSA, som idag kallas boskaps-relaterad MRSA (”Livestock associated”, LA-MRSA). Den skiljer sig genetiskt från de typer man isolerat tidigare, såsom sjukhus-relaterad MRSA (”healthcare associated”, HA-MRSA) och samhälls-relaterad MRSA (”community associated”, CA-MRSA). När den boskapsrelaterade MRSA:n påvisades upptäcktes att den i hög grad spreds till de människor som hanterar djuren, såsom djurskötare, ägare och veterinärer. EU har

uttryckt att de anser att detta innebär en stor risk som kan ge direkta konsekvenser för allmänhetens hälsa. I västra Europa har många studier gjorts på förekomsten av MRSA hos grisar och de flesta visar på en mycket hög förekomst, speciellt i länder som har stora grisbesättningar. I Danmark har det visat sig att upp till 89 % av grisarna bär på MRSA, och bland de infektioner som drabbar människor står den boskapsassocierade MRSA:n för 35 %. I Belgien har studier visat att 87–94 % av grisarna bär på MRSA, medan i Tyskland och Spanien ses en något lägre förekomst, 41 % respektive 51 %. Sverige anses vara fritt eller ha mycket låga nivåer av MRSA hos sina grisar, då man endast hittat MRSA en gång, nämligen 2014. Det var då enbart ett fåtal grisar som bar på bakterien och studier som gjorts efter detta har inte hittat någon MRSA hos gris.

I Uganda har mycket få studier gjorts på MRSA både inom human- och djurnivå och det är därför väldigt svårt att uppskatta förekomsten av MRSA. Enstaka studier som gjorts på grisar visar en hög andel positiva djur, runt 65 %, men då dessa har gjorts på ett fåtal grisar är inte resultaten pålitliga. Något man dock bör ha i åtanke är att i Sverige har vi en mycket strikt antibiotikapolicy vid behandling av djur och man får t ex inte ge antibiotika för att djuren ska växa bättre, vilket är vanligt förekommande utanför EU. I Uganda finns också lagar som förhindrar fri användning av antibiotika, men trots detta finns antibiotika tillgängligt på de flesta apotek, både för mänsklig och veterinär bruk. Utöver detta är kunskapsnivån låg och många vet inte varför, hur och när antibiotika korrekt bör användas.

I min undersökning besöktes tjugo olika grisägare som intervjuades kort innan slumpvalda grisar i rätt ålder provtogs med hjälp av provtagningstops. Totalt togs 50 prov hos 19 djurägare. Dessa prov odlades med hjälp av en metod som är speciellt utarbetad för just MRSA samt med en metod som var mindre selektiv. Den första odlingen gav upphov till en misstänkt och en konstaterad MRSA, varav den misstänkta stammen senare visade sig vara en annan typ av stafylokock. Vid den andra, mindre selektiva odlingen hittades ytterligare arton misstänkta stafylokocker, varav tre var misstänkta *S. aureus*. Dessa tjugo prover skickades till Sverige och analyserades med MALDI-TOF för att typa stafylokocker. Därefter gjordes en analys av huruvida bakterien var resistent eller inte genom att bakterien blandas med buljong och antibiotika i bestämda koncentrationer som inkuberas i lämplig temperatur under en viss tid. Därefter bedöms vid vilka koncentrationer som bakterien kunnat växa, eller ej. Dessa värden jämförs sedan med vedertagna gränsvärden för om en bakterie skall klassas som känslig eller resistent. Alla prover i studien utom två var resistenta mot någon typ av antibiotika och totalt fyra prover, inklusive MRSA:n, var så kallade multiresistenta bakterier, vilket innebär att de är resistenta mot minst tre olika klasser av antibiotika.

Eftersom skillnader i hur man håller och hanterar djur i till exempel Afrika och Europa förekommer är det svårt att dra paralleller till och jämföra med de europeiska sifforna. Danmark har en väldigt hög förekomst av MRSA hos sina grisar, nästan 90 %, till skillnad från Sverige där MRSA bara påvisats enstaka gånger och hos enstaka grisar. I Europa varierar förekomsten av MRSA och genomsnittet för hela EU är 26,9 %. De besättningar vi besökte hade ofta få djur som gick utomhus och man var oftast inte så noggrann med kontakter med andra grisar. Man köper och säljer grisar till varandra och flyttar dem mellan gårdar utan att hålla dem i karantän. I Sverige är många grisgårdar slutna och vem som helst får inte komma in, och vid tillträde

krävs skyddskläder i form av gårdens egna stövlar och overaller eller engångskläder. När nya grisar tas in hålls de oftast i karantän för att förhindra att några nya smittor sprids till resten av grisarna. I Sverige behandlar man endast med antibiotika när situationen så kräver, medan intrycker av intervjuerna vi gjorde var att man i Uganda ibland behandlade lite ”på känn”. De flesta ringde enligt utsago veterinär när grisarna var sjuka, men vissa behandlade på rutin och ”på känn”. Antibiotika är också väldigt lättillgängligt i Uganda, vi gick och frågade på ett veterinär-apotek, som då direkt erbjöd oss flera olika sorter utan att kräva recept eller legitimation.

Resultaten av denna studie visar att förekomsten av MRSA hos små grisbesättningar i Lira, Uganda är 5 %. Metoden jag använt anses vara bra, men eventuellt ger den något högt falskt negativa prov, det vill säga att den kan missta en MRSA för en vanlig *S. aureus*. Oavsett detta är andelen MRSA väldigt låg. Man bör vara försiktig med att dra allt för många slutsatser från detta resultat, till exempel är proven tagna i besättningar med relativt få djur (upp till 20), och det är därmed svårt att säga hur det skulle se ut i större besättningar. Utöver detta är mitt urval litet, och totalt har 87 grisar provtagits och poolats till 51 prov. I de studier som gjorts i Europa är oftast prover tagna från hundratals grisar.

Något övrigt att lära sig från studier som den här är utmaningen det innebär att utföra en studie i ett främmande land, tillika ett låginkomstland. Det finns många möjliga felkällor, såsom problem med att göra sig förstådd, trots närvaro av tolk, okänd utrustning eller utrustning av annan sort än den man är van vid, sämre elförsörjning och frånvaro av korrekt kylförvaring. Hygienstandarden är sämre jämfört med i Sverige, vilket givetvis ger upphov till en stor potentiell risk för felkällor.

Sammanfattningsvis kan man konstatera att trots användningen av antibiotika i Uganda är stor och dåligt kontrollerad, och trots att de har sämre förutsättningar för att undvika att smittor och bakterier sprids mellan djuren, så hittades en väldigt låg förekomst av MRSA. Fler och större undersökningar skulle behöva göras för att dra en säker slutsats.

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APPENDIX I

Questionnaire

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?