A pilot study of the epidemiology of *Staphylococcus aureus* in multiple site swine production

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Dedication

This thesis is dedicated to my husband, Daniel Linhares. Thank you for all the love and support that you provide to me during this Master program. I am really proud of being your wife and partner and I dedicate this work to you.

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Introduction

Staphylococcus aureus (S. aureus) is a common colonizer of both humans and pigs (Lowy, 1998; Frana, 2012). The ability of *S. aureus* to acquire genes that confer resistance to multiple drugs has further elevated its importance to public health (Cuny and Witte, 2008a). In particular, clones of *S. aureus* that are resistant to methicillin and other beta-lactam antimicrobials (MRSA) are a major clinical problem, and the discovery of MRSA in livestock populations has raised concerns about the potential importance of livestock as reservoirs of MRSA (Voss et al., 2005).

However, the importance of pigs in *S. aureus* transmission to humans and clinical disease is yet to be determined (Cuny and Witte, 2008b). Most recent studies of pigs have focused on MRSA, and there have been no comprehensive studies of the epidemiology of *S. aureus* (MSSA and MRSA) in pigs.

Despite being considered ubiquitous in production animal facilities (Frana, 2012), *S. aureus* ecology in livestock production farms is poorly documented. Most recent research has used selective enrichment methods to study MRSA in swine populations, rather than generic *S. aureus*. *S. aureus* can be isolated from several anatomic sites of pigs, as well as from air, environmental samples and persons having contact with pigs. In fact, isolation of *S. aureus* in air samples from swine barns suggests this is likely an important route of exposure for people working in livestock facilities (Gibbs et al., 2006; Oppliger et al., 2012). Overall, the limited information on the ecology of *S. aureus* in the pork production chain limits the ability of the swine industry to understand and communicate the risks to public health in an informed manner.

The core rationale for this thesis was that there has been no prior systematic effort to describe the occurrence of *S. aureus* in swine production systems. The vast majority of studies have focused on MRSA strains using selective culture methods, and/or focused on a limited number of matrices. The objective was therefore to obtain preliminary data on the occurrence of *S. aureus* in pigs, people, environmental and air samples on pig farms and some insight into the distribution of the organism in the swine farm milieu. Thus, a pilot study of the epidemiology of *S. aureus* in multiple site swine production was conducted.

Chapter 1 – Literature review.

Staphylococcus aureus

S*taphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium belonging to the class Bacilli, order Bacillales and family Staphylococcaceae. It was first identified in an abscess of a human knee joint by Sir Alexander Ogston in Scotland in 1880 (Macdonald and Smith, 1984).

Considered part of the normal bacterial flora of many mammalian and avian species, *S. aureus* colonization is typically not associated with infection, but in humans constitutes an importance source for opportunistic infections of the bloodstream, skin and soft tissue, as well as nosocomial pneumonia (Lowy, 1998). Similarly, it is generally accepted that *S. aureus* is commonly found as commensal organism in pigs and other animals, and that occasionally it might cause skin and soft tissue infections (Frana, 2012).

Methicillin resistant *Staphylococcus aureus* in humans and pigs

Methicillin resistant *Staphylococcus aureus* (MRSA) are a specific class of *S. aureus* that has been described since 1961 (Leonard and Markey, 2008). Methicillin resistance results from acquisition of a gene called mecA that encodes the PBP2 protein, which has low affinity for beta-lactam antibiotics (Vanderhaeghen et al., 2010). The mecA gene is located within a mobile genetic element called the staphylococcal chromosomal cassette, or SCCmec (Berger-Bächi and Rohrer, 2002). As a result, MRSA are often multi-resistant to several antibiotics.

Until the 1990's MRSA was perceived as a pathogen largely limited to humans, and animal reservoirs were considered of negligible epidemiological significance for human

staphylococcal infections. Furthermore, clinical MRSA infections of people were predominantly confined to hospitals (i.e. 'hospital acquired' MRSA, or HA-MRSA) (Morgan, 2008). An important shift in MRSA epidemiology occurred during the mid 90's with the global emergence of community-associated MRSA (i.e. CA-MRSA), a phenomenon that was described as a '*quantum change in the biology and epidemiology of a major human pathogen*' (Diep and Otto, 2008).

Subsequently there have been increasing reports of MRSA in animals and reports of apparent transmission between humans and animals, which have raised questions about the significance of animal reservoirs of MRSA as a source of human infections (Voss et al., 2005; Leonard and Markey, 2008; Morgan, 2008; Wulf and Voss, 2008; van Cleef et al., 2010b). In particular, the recognition of the ST 398 lineage of MRSA in livestock in Europe has raised concerns that food animals could constitute an important reservoir for human infection (Voss et al., 2005).

S. aureus virulence factors

The propensity of *S. aureus* to acquire genes that confer to resistance to antimicrobials particularly to beta-lactam antibiotics has become a major problem for treatment of *S. aureus* infections (Utsui and Yokota, 1985). In addition to antibiotic resistance, as reviewed elsewhere, there are several known virulence factors produced by *S. aureus*, including hemolysins, DNAase, lipase, protein A, coagulase, staphylokinase, leukocidin, hyaluronidase, techoic acids, and several toxins including enterotoxins, pore-forming toxins and exfoliative toxins (Chua et al., 2013; Grumann et al., 2013; Zecconi and Scali,

2013).

S. aureus-produced toxins of great public health importance are the toxic shock syndrome toxin-1 (TSST-1) and enterotoxins. TSSTS-1 causes endothelial toxicity and has superantigen activity, which causes non-specific T-cells and massive cytokine release (Zecconi and Scali, 2013). Enterotoxins may harm infected hosts due to immunomodulation via superantigen activity resulting in gastroenteric toxicity (Peacock et al., 2002). It is noteworthy that *S. aureus*-produced enterotoxins can be isolated from food products and thus cause food intoxication, highlighting the importance of hygiene control in the food supply (Peeva and Gogov, 1983).

S. aureus typing

There are numerous methods used for subtyping of *S. aureus*. Below is the summary of the most common methods used to type *S. aureus*, which include PFGE (digestion enzymes of the whole-cell DNA), and DNA sequence-based methods of multilocus sequence typing (MLST) and typing of the *S. aureus* protein A (spa) gene.

Pulsed-field gel electrophoresis (PFGE)

PFGE is the traditional gold standard method use to type *S. aureus* for clinical epidemiology. PFGE employs restriction enzymes that cleave *S. aureus* whole DNA at different loci defined by base pair sequences, thereby generating DNA fragments of

different band sizes (50 to 700Kb) and patterns that can be visualized using gel electrophoresis (Schouls et al., 2009). Types are defined according to the different band patterns (Mulvey et al., 2001).

PFGE is a simple, straightforward method to compare isolates and there is a large database accumulated since this method is used by the U.S. Centers for Disease Control and Prevention (CDC). However, it is time consuming (2-6 days) and there is a poor inter-lab agreement when compared to DNA sequencing-based methods (Tenover et al., 1994).

It is important to mention that new strains of *S. aureus* can emerge that are not typable with PFGE depending on the set of enzymes used. In fact the initial detection of the ST398 lineage of MRSA that has been associated with livestock resulted from the inability to digest the DNA with the sma1 enzyme used routinely for PFGE in the Netherlands. (van Loo et al., 2007). Currently the CDC also uses and recommends routine PFGE with SmaI enzyme, and that for isolates that are not typable with this enzyme, EagI or its isoschizomer, Cfr9i should be used [1].

Multilocus Sequence Typing (MLST)

DNA is extracted from the sample and submitted to a set of PCR assays. The products of those PCRs are then submitted to automated DNA sequencing from 7 known housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi and yqiL)[2]. The information from the

[1] http://www.cdc.gov/hai/pdfs/labSettings/Unified_PFGE_Protocol.pdf
[2] The MLST housekeeping genes that are sequenced are carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glpF), guanylate kinase

sequences is analyzed by software called eBURST (http://saureus.mlst.net/eburst) and the pattern of the sequences can be compared using an online database (http://saureus.mlst.net). As of May 30th, 2013 this database had catalogued 2,523 *S. aureus* subtypes based on MLST patterns. *S. aureus* subtypes based on MLST typically use the "ST" (sequence type) designation followed by a number. For example, *S. aureus* isolates identified in pigs are frequently typed as ST 398, the first MLST typed defined to be associated with livestock.

MLST is considered as having high discriminatory power. However, it is a relatively expensive method, as it requires access to a high-throughput DNA sequencing facility.

Spa typing

S. aureus bacteria possess the staphylococcal protein A (spa) which can bind to immunoglobulins and is considered a virulence gene. The gene that encodes the spa protein includes the X-region, which consists of a segment with a varying number of small (21 to 27 bp) repeats (Frénay et al., 1996). Based on the repeat pattern, isolates can be classified into different "spa types". To ensure consistency in the genotyping there are computerized methods using unique algorithms to assign a genotype according to the pattern of repeats (Mellmann et al., 2007). One example is the Ridom spa server available at the website "http://spaserver.ridom.de" (Harmsen et al., 2003). As of May 30th, 2013

(gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi) and acetyl coenzyme A acetyltransferase (yqiL)

there were 265,474 isolates from 52 countries listed on the Ridom server, representing 12,325 spa types.

Spa typing methods are cheaper (only one PCR reaction needed) and also are generally more discriminatory than MLST (seven PCR reactions). Typically, many spa types can occur within a single MLST type (e.g. t011, t571, t567 belong to ST398), but the same spa type can occur in different MLST types.

A second system to type *S. aureus* based on the spa gene variability is the BioNumerics software (http://www.applied-maths.com/bionumerics). BioNumerics uses the multiple-locus variable-number tandem-repeat analysis (MLVA) method (described below) to subtype *S. aureus* isolates based on the variable copy numbers of tandem repeats (VNTR) of the spa gene. Similar to the Ridom platform, there is a worldwide database of BioNumeric spa types. The Ridom spa server is a freely accessible platform and BioNumerics is private. A third tool is the eGenomics server (www.egenomics.com), which is a paid service that generates spa types. The BioNumerics and eGenomics spa types are designated using a numerical system (e.g., spa type 539) while Ridom spa types are designated using a numerical system preceded by a 't' (e.g., t034).

Advantages of spa typing include good predictive power and reliable intra- and inter-laboratory reproducibility since it is a sequence based method evaluated by standard algorithms (Aires-de-Sousa et al., 2006). Disadvantages include the fact that it requires an automated sequence to be inserted on an online server.

Multiple-locus variable-number tandem-repeat analysis (MLVA)

PFGE, MLST and spa typing have great discriminatory power and are the most commonly used methods to categorize *S. aureus* isolates. The extensive data available in the literature on *S. aureus* that have been genotyped using MLST and spa typing methods allow comparisons of isolates from different geographic locations. However, those are relatively expensive and laborious approaches. Multiple-locus variable-number tandem-repeat analysis (MLVA) has been proposed as a novel method to genotype *S. aureus* , which is based on the analysis of the variation in number of repeats in seven individual genes (sspA, spa, sdrC, sdrD, sdrE, clfA, and clfB) (Malachowa et al., 2005). MLVA is performed using a multiplex PCR targeting the aforementioned genes and has proven to have similar (but lower) discriminatory power to PFGE and higher discriminatory power than spa typing (Malachowa et al., 2005; Schouls et al., 2009). Compared to PFGE, MLST and spa typing, MLVA is a rapid, simple and relatively cheap method to genotype *S. aureus*. However, there is limited information of *S. aureus* types based on MLVA and thus this method deserves further validation.

SCCmec typing

MRSA isolates can be differentiated using PCR-based assays to analyze the staphylococcal cassette chromosome *mec* (SCC*mec*), which includes the *mec*A gene that confers methicillin resistance (Katayama et al., 2000). *SSCmec* is composed of 2 essential components, the *ccr* gene complex (ccr) and the mec gene complex (mec). Based on the

allotypes of these two components, SSC*mec* can be further classified into at least 11 types (I-X1) (Ito et al., 2004; Turlej et al., 2011).

Classification according to antibiotic resistance

S. aureus was an important bacterium before the antibiotic era, with first description of a clinical case in the 1880s (Macdonald and Smith, 1984). With the advent of antibiotics, *S. aureus* infections were effectively treated with common antibiotics such as penicillin. However, since the early 1960's, a specific lineage of *S. aureus* acquired resistance to methicillin and since then it has become a convention to categorize the organism accordingly into methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) (ERIKSEN and ERICHSEN, 1963). MRSA are frequently multi-drug resistant and thus of higher public health concern than MSSA (Cuny and Witte, 2008a).

Further molecular characterization of MRSA isolates showed distinct lineages of closely related MRSA among the livestock-associated, hospital-associated or general community-associated isolates (LA-MRSA, HA-MRSA and CA-MRSA respectively) (Enright et al., 2002; Voss et al., 2005; Smith and Pearson, 2011). LA-MRSA was found in production animals and in people with occupational exposure to such animals (van Cleef et al., 2010a; van Cleef et al., 2010b). LA-MRSA isolates from pigs are generally subclinical (healthy pigs) and classified as sequence-type (ST) 398 based on MLST. In humans, patients with ST398 versus all other MRSA had significantly shorter length of stay in hospitals and were less likely to be admitted to intensive care units (Köck et al., 2011). Other livestock-associated *S. aureus* strains reported in pigs include ST 9 which appears to be predominant in several Asian countries (Cui et al., 2009; Guardabassi et al.,

2009; Neela et al., 2009), and ST 97 in Europe and the USA (Battisti et al., 2010; Osadebe et al., 2013) and ST 5 in Canada and USA (Khanna et al., 2008).

The emergence of LA-MRSA in pig populations is a valid cause of concern and thus public health implications need to be better understood. However, in approximately eight years since being first recognized, the burden on human health has been minor, and the risk of exposure to these organisms is overwhelmingly concentrated in people with occupational exposure to livestock (Davies, 2012).

S. aureus biology in humans

The major route of *S. aureus* excretion in humans is via the respiratory route (WILLIAMS, 1963; Wertheim et al., 2005) and the most prevalent sites of colonization in the adult population are the nares, throat, perineum and skin (Sollid et al., 2013). In pigs there are reports of *S. aureus* isolation from different anatomical sites including skin (Akatov et al., 1983), tonsils (Skalka, 1991; Zhang et al., 2012), feces (Dimitracopoulos et al., 1977; Friese et al., 2013), nose (Frana et al., 2013), internal organs (Skalka, 1991; van der Wolf et al., 2012) and arthritic joints (Turner, 1982). Likewise, *S. aureus* has been isolated from pigs of different age groups, that is, from newborn to adult age pigs (Crombé et al., 2012; Hawken et al., 2013).

Epidemiology in pig farms

There are other several staphylococcal species found in pigs including *S. chromogenes, S. epidermidis, S. hyicus, S. sciuri, S. warneri* and *S. xylosus*. However, only *S. hyicus* and *S. aureus* are considered potentially pathogenic to pigs. The former causes exudative epidermidis and the latter abscesses, besides being associated with other conditions including septicemia, mastitis, vaginitis, metritis, osteomyelitis, and endocarditis (Frana, 2012). However, it is generally accepted that *S. aureus* is an ubiquitous opportunistic bacteria as opposed to the primary cause of the aforementioned conditions (Frana, 2012).

Some suggested risk factors associated with higher MRSA prevalence and transmission between and within pig populations, include:

- Pig introduction – introduction of pigs from MRSA-positive herds increase the likelihood of a farm having MRSA-positive pigs (direct contact)(van Duijkeren et al., 2007);
- Environmental contamination, presence of other livestock and farm management (internal biosecurity measures) (Alt et al., 2011; Crombé et al., 2013)
- Herd size - larger herds are more likely to have constant pig introductions, and have a higher number of susceptible pigs by birth or purchase and therefore have higher risk of having harboring-MRSA pigs (Alt et al., 2011; Broens et al., 2011b).

- Type of pig production – outdoor-reared pigs having lower pressure of infection and therefore may be at lower risk of being S. aureus carriers than pigs reared on indoor operations (Porrero et al., 2012).

- Proximity to other livestock production facilities – the higher the density of livestock farms neighboring the farm, the higher the chances of having MRSA detected in the air favoring airborne spread (Schulz et al., 2012).

S. aureus has been previously reported in the air (Elliott et al., 1976; Gibbs et al., 2004; Friese et al., 2012; Schulz et al., 2013), on environmental samples of pig barns (Raszyk, 1986; Friese et al., 2013) and from persons that had contact with pig farms (Osadebe et al., 2013). However, to date no studies have evaluated prevalence of *S. aureus* among all those different matrices within a swine production system.

One study showed that adult pig females (sows) had *S. aureus* prevalence similar to that of their respective piglets (Nathaus et al., 2010), and another study reported an increasing prevalence of *S. aureus* from suckling pig-age until finishing-age (Weese et al., 2011). However, Hawken and colleagues conducted a longitudinal study of MRSA and reported decreasing prevalence in pig nasal samples over time (Hawken et al., 2013). It is possible that herd specific patterns of transmission may occur and therefore variation in results among studies is not surprising.

Altogether, those studies contribute to understanding *S. aureus* epidemiology in pigs. However, there has been no systematic study to evaluate sampling protocols for isolating *S. aureus* from pigs. Such fundamental studies are required to understand the relative sensitivity of detection, and possible sampling biases associated with arbitrary sampling of individual anatomical sites or age groups.

Transmission between pigs and humans

S. aureus is a common colonizer of both humans and pigs (Lowy, 1998; Frana, 2012). However, the role of pigs in *S. aureus* transmission to humans is yet to be determined (Cuny and Witte, 2008b). The limited information on the ecology of S. *aureus* in the pork production chain limits the ability of the swine industry to understand and communicate the potential risks to occupational and public health in an informed manner.

It has been suggested that pigs play an important role in the transmission of *S. aureus*, including MRSA, to humans (Lee, 2003; Huijsdens et al., 2006; Wulf and Voss, 2008).

In fact, several studies in multiple countries (Singapore, Germany, United States, Canada, China, Malaysia and Ireland) have reported similar prevalence of MRSA in pigs and humans with occupational exposure to pigs, most of them with similar spa types between pigs and humans (Sergio et al., 2007; Khanna et al., 2008; Meemken et al., 2008; Cui et al., 2009; Neela et al., 2009; Smith et al., 2009). It is important to highlight that not all pig herds and farm workers carry MRSA. Horgan and colleagues sampled 440 pigs and 101 humans from 41 farms in Ireland and found no LA-MRSA (Horgan et al., 2011). Recently Smith and colleagues detected MRSA in only 4 of 45 herds in the USA, and comparable herd prevalence (5 of 46 farms) was also reported from Canada (Weese et al., 2011; Smith et al., 2013).

As pointed out by Crombé and colleagues, caution must be taken when comparing prevalence from different studies due to differences in sampling, isolation procedures, number and type of pigs sampled and sample populations (finishing vs. breeding pigs,

piglets vs. older pigs, open vs. closed farms, pigs at the abattoir vs. pigs at the farm, etc. (Crombé et al., 2013).

A study in Spain reported a similar MRSA isolate found on a skin lesion of a pig farmer, members of his household and pigs from his pig farm. Isolates from pigs and humans shared the same MLST sequence type, spa type (ST398-t1451), antibiotic resistance profiles (tetracycline, erythromycin and clindamycin resistances and harbored the tetK, tetM, and ermC resistance genes) and had closely related PGFE patterns. Therefore, that case report suggests a possible MRSA transmission between pigs and humans (Lozano et al., 2011). However, overall there are few reports of clinical infection in people exposed to pigs. Furthermore, most studies have been cross-sectional and have not permitted evaluation of whether people who are culture positive for ST398 *S. aureus* are permanently colonized or transiently contaminated with these organisms.

Diagnosis and treatment

Diagnosis of *S. aureus* consists of culturing and isolating it from samples, followed by a typing method if molecular epidemiology is necessary. It has been proposed that Mueller–Hinton broth with 6.5 to 7.5% NaCl (MHB+)-enriched wipes can be used as a sampling tool to screen the pig farm environment for the presence of MRSA or MSSA *S. aureus* (Broens et al., 2011a) as staphylococci are some of the few microorganisms that can tolerate such high levels of salt in the broth. Additionally, mannitol is a commonly used media in the process of *S. aureus* isolation, as fermentation of the sugar by *S. aureus* causes a shift in the pH, turning the indicator from red to yellow. Furthermore, *S. aureus*

is produces the enzymes catalase and coagulase, which can be readily detected using standard commercial kits. These routine methods facilitate *S. aureus* selection and identification during the bacterial isolation process (Cowan and Steel, 1965).

S. aureus can be cultured by direct plating on solid media, with or without prior enrichment. A common enrichment procedure for isolating *S. aureus* is to incubate the sample at 35-37ºC for 24 hours under aerobic conditions in Mueller-Hinton broth enriched with 6.5% NaCl, followed by incubation on phenol-red mannitol broth for additional 24h at 35-37°C. Then, when the broth changes color, a loop of sample can be streaked to a columbia-CNA agar plate and incubation at 35-37°C for 24 hours. *S. aureus* colonies are typically round-shaped with an opaque golden-yellow color, with hemolysis on sheep-blood agar. To confirm as *S. aureus* one might use the tube-coagulase test and the *S. aureus* latex agglutination assay (Cowan and Steel, 1965).

Summary

S. aureus is a common bacterium which colonizes humans, pigs and other production animals. Furthermore, there is considerable evidence to suggest that interspecies transmission of *S. aureus* does take place. *S. aureus* has the potential to acquire antimicrobial resistance genes (MRSA) and cause opportunistic infections.

It is important to emphasize that most of the recent work on *S. aureus* has focused on MRSA, with very few studies looking at MSSA. Notwithstanding, it is important to

understand the epidemiology of *S. aureus* in pigs generally (MSSA and MRSA), in order to be able to interpret information about MRSA.

Understand the ecology of *S. aureus* in pig farms around the world is limited by the paucity of comprehensive studies designed to understand the biology of organism on farms. Basic needs relate to identifying the optimal sample type (air, dust, pig, farm workers) for detecting *S. aureus* at the animal, group or herd levels, and the effects of pig age and anatomic site. The work undertaken in this thesis was designed to provide preliminary data on these basic questions of *S. aureus* biology on commercial swine farms in the USA.

Chapter 2 – Effect of anatomic site and age on detection of *S. aureus* in pigs

Introduction

S*taphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium belonging to the Bacilli class, Bacillales order and Staphylocaccaceae family. It was first identified by Sir Alexander Ogston in Scotland in 1880 in an abscess of a human knee joint (Macdonald and Smith, 1984). The emergence of methicillin-resistant *S. aureus* (MRSA) in the early 1960's had important clinical ramifications. (ERIKSEN and ERICHSEN, 1963). Subsequently it has become a convention to distinguish MRSA from methicillin-susceptible *S. aureus* (MSSA) in most clinical and epidemiological reports. MRSA are often multi-drug resistant bacteria, further increasing the risk of treatment failure and therefore public health concern (Utsui and Yokota, 1985; Enright et al., 2002; Crombé et al., 2013).

S. aureus is a common colonizer of both humans and pigs (Lowy, 1998; Frana, 2012). However, the importance of pigs in *S. aureus* transmission to humans and clinical disease is yet to be determined (Cuny and Witte, 2008b). Most recent studies of pigs have focused on MRSA, and there have been no comprehensive studies of the epidemiology of *S. aureus* (MSSA and MRSA) in pigs. This limits the ability of the swine industry to communicate the risks of *S. aureus* to public health in an informed manner.

There are numerous reports of isolation of *S. aureus* from pigs and it is generally assumed that, analogous to the situation in humans, *S. aureus* is part of the normal bacterial flora of swine (Frana, 2012). Anatomical sites from which *S. aureus* has been isolated include skin (Akatov et al., 1983), tonsils (Skalka, 1991; Zhang et al., 2012), feces (Dimitracopoulos et al., 1977; Friese et al., 2013), nose (Frana et al., 2013), internal

organs (Skalka, 1991; van der Wolf et al., 2012) and arthritic joints (Turner, 1982). *S. aureus* has also been isolated from various age groups of pigs from newborn to adult (Crombé et al., 2012; Hawken et al., 2013). However, there has been no systematic study describing patterns of colonization in swine populations or comparing sampling protocols for detecting *S. aureus*.

Therefore, this study was undertaken to (a) compare the effect of anatomical site on detection of MSSA and MRSA *S. aureus* in pigs, and (b) compare *S. aureus* prevalence in sows, suckling pigs, weaned pigs and finishing pigs.

Design and methods

Study design and source population

A longitudinal study was conducted in 2 multiple site pig production systems located in Minnesota, United States. The farms were selected by convenience based on willingness of the producers to participate and proximity. Sampling was conducted in pigs in the farrowing rooms at the breeding herd sites, and during the nursery and finishing phases at growing pig sites. Two cohorts were sampled in each system, with an interval of at least 6 months between cohorts. At each breeding herd site, samples were collected from lactating sows (n=12) in rooms housing the youngest litters, and samples were also collected from 1 piglet per litter of the sows sampled (n=12). Subsequent sampling of the same birth cohorts of pigs occurred 4 and 20 weeks later at "nursery" (n=12) and "finishing age" (n=12) phases of pig production, respectively. Swab samples were

collected from the nose, tonsil, feces, and skin (axilla) of each pig. Additionally, vaginal samples were collected from sows.

Animal sampling

Using sterile swabs (BBL CultureSwab[e], Liquid Stuart medium single plastic applicator, Becton, Dickinson and Co., Sparks, MD, USA) nasal, tonsil, fecal, skin (axilla) and vaginal samples were collected from 192 pigs. To collect nasal samples, a single swab was inserted 2 to 7 cm (according to pig size) into both nostrils and gently rotated against the mucosal epithelium. To enable collection of tonsillar samples, a speculum was used to open the mouth, and the swab was gently rotated against the tonsillar epithelium. The skin sample was obtained by rubbing a swab onto the axillary folds of the pigs. The fecal sample was obtained by introducing a swab (about 5 cm) into the rectum of pigs and gently rotating the swab in the rectal contents. For vaginal sampling, a swab was gently rubbed against the vaginal epithelium using circular rotations introducing a swab 5cm into the vagina. Following sample collection the swabs were individually identified and stored on ice until processed in the laboratory.

All procedures with animals were approved by the University of Minnesota animal care and use committee (IACUC) by the protocol number 1105B99155.

Isolation and Identification of S. aureus *bacteria*

All samples were cultured in parallel by two culture procedures, described as selective (specific for culture of MRSA) and non-selective enrichment techniques (MSSA). All swabs were placed in 15ml tubes (BD Falcon 15ml Conical Centrifuge tubes, Fisher

Scientific Inc., Waltham, MA) with 5 ml of Mueller-Hinton broth (Teknova, Hollister, CA) containing 6.5% NaCl (Sigma-Aldrich, Saint Louis, MO) and incubated at 37°C for 24 hours.

The procedure to isolate MSSA consisted of transferring a 1 ml aliquot from the Mueller-Hinton broth into 9ml of phenol-red mannitol broth (Becton Dickinson, Franklin Lakes, NJ). After 24h incubation at 37°C, if the broth changed color, one 10µl-inoculating loop of broth was streaked onto a BBL Columbia-CNA agar plate with 5% Sheep Blood (Becton Dickinson, Franklin Lakes, NJ). The plates were incubated at 37°C for 24 hours. Two presumptive *S. aureus* colonies (round, golden-yellow, colonies with hemolysis) were then each restreaked onto new Columbia-CNA plates. The plates were incubated at 37°C for 24 hours. Once pure cultures were obtained, all suspected isolates were confirmed as *S. aureus* using the tube-coagulase test (Difco, Detroit, MI) and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad).

The procedure to isolate MRSA consisted of placing 1ml aliquot from the Muller-Hinton broth into a 9 ml Phenol-Red Mannitol broth (Becton Dickinson, Franklin Lakes, NJ) containing 4mg/L oxacillin (Sigma-Aldrich, Saint Louis, MO). Samples showing color change indicating mannitol fermentation after 24h incubation at 37°C, were streaked (10µl-inoculating loop) onto a chromogenic agar plate (MRSAselect^{TM}, Bio-Rad, Hercules, CA) and incubated at 37°C for 24 hours. Presumptive methicillin resistant *S. aureus* colonies (purple/blue on the white/cream media) were to be restreaked onto Columbia-CNA plates and incubated at 37°C for 24 hours. Once pure cultures were obtained, all suspected isolates were to be confirmed as MRSA using the tube coagulase

test (Difco, Detroit, MI) and by testing for the presence of penicillin binding protein2a using a commercial latex agglutination test (MRSA latex agglutination test, Oxoid Ltd., Hants, UK).

Outcomes and statistical analysis

This study investigated the effects of 'anatomical sampling site' (nose, tonsil, feces, and skin or vagina) and 'pig age group' (sow, preweaning piglet, nursery age and finishing age) on *S. aureus* prevalence, after adjusting for correlations at the individual pig, cohort and farm levels.

The comparison of *S. aureus* prevalence between the different pig-age groups was performed at two levels: pig level and sample level. At the pig level, a pig was considered *S. aureus*-positive if at least one anatomical site was culture positive for *S. aureus*. At the sample level, all samples (i.e., from all anatomical sites) contributed to the prevalence calculation.

The statistical analyses were performed with SAS package version 9.3 (SAS Institute Inc., Cary, NC) using generalized mixed models (Proc Glimmix), considering pig nested within cohort nested within farm (random effects). More specifically, the Proc Glimmix was used to model the probability of a sample being *S. aureus* positive using a binary response distribution with a logit link function.

Results

No MRSA were detected in any samples from this study; therefore all isolates herein reported were MSSA.

Effect of age group on S. aureus prevalence

The prevalence of *S. aureus*-positive pigs (pig level analysis) did not differ among age groups (P = 0.55) and was 96%, 92%, 90% and 88% in finishing-age, nursery-age, sows and suckling piglets respectively (Table 1). At sample level, prevalence of positive samples was highest in finishing-age pigs, followed by nursery-age pigs and suckling piglets and sows (Table 2). There was no difference of *S. aureus* prevalence in samples from suckling piglets and sows (P = 0.62). However, the prevalence of *S. aureus* in nursery pig samples was significantly higher than in samples of sows or preweaning piglets (P < 0.0001 and P < 0.0001, respectively) and lower than that of samples from finishing-age pigs (P = 0.016).

Effect of anatomical site on detection of S. aureus

The proportion of samples positive for *S. aureus* varied among anatomical sites (p-value <0.001). Prevalence was higher in nose (68%), skin (62%) and tonsil (62%) samples compared to fecal (42%) and vaginal (40%) samples (Table 3).

Discussion

Although *S. aureus* is generally considered to be 'normal flora' of pigs, there is a dearth of studies that describe the ecology of *S. aureus* in swine populations. In addition to *S. aureus*, there are several staphylococcal species reported to occur in pigs including *S. chromogenes, S. epidermidis, S. hyicus, S. sciuri, S. warneri* and *S. xylosus*. However, only *S. hyicus* and *S. aureus* are considered potentially pathogenic to pigs. *S. hyicus* is the primary agent of exudative epidermidis in pigs, although there are some reports implicating *S. chromogenes* and MRSA in this condition (Andresen et al., 2005; van Duijkeren et al., 2007). *S. aureus* is an opportunistic pathogen of pigs that is often isolated from abscesses, and can be found in cases of septicemia, mastitis, vaginitis, metritis, osteomyelitis, and endocarditis (Frana, 2012).

In a broad review of the ecology of staphylococci in humans and animals, Kloos stated that mammalian skin (including the anterior nares) is a major habitat of staphylococci, and that staphylococci also occur in other regions of the body such as the throat, mouth, mammary glands, and intestinal tract, albeit less frequently or in smaller numbers than on skin (Kloos, 1980). The same author also observed that staphylococcal populations of pigs were not well characterized. Active research of *S. aureus* in swine has been recently initiated since the discovery that MRSA variants occur commonly in pigs (Voss et al., 2005). However, systematic investigation of the ecology of *S. aureus* has not previously been conducted.

Overall, the observations made in the 2 herds investigated in this study supported the contention that *S. aureus* constitutes part of the normal bacterial flora of pigs.

Approximately 90% of pigs sampled in all age groups were culture positive for *S. aureus* in at least one anatomical site. However, a higher proportion of anatomical sites were positive in growing pigs than in sows and suckling pigs. A study comparing nasal and tonsillar flora of pigs found *S. aureus* and most lactobacilli became more prevalent after weaning (Baele et al., 2001). Previous studies using nasal swabs to detect MRSA in swine herds have also suggested that the prevalence of positive swabs may be higher in growing pigs than suckling pigs (Nathaus et al., 2010; Broens et al., 2012).

In contrast, Hawken and colleagues reported a decline in MRSA prevalence in nasal swabs of growing pigs with prevalence highest in nursery aged pigs (Hawken et al., 2013). It is important to note that these few studies have been conducted on small numbers of farms and there is evidence for variation among farms (Broens et al., 2012).

The present data indicate that the nose, tonsils and skin are the anatomical sites most likely to be culture positive for *S. aureus* in pigs. However, *S. aureus* was also prevalent in swine feces and in vaginal swabs of sows. However, as the pigs in these commercial units are housed in groups it is impossible to determine whether these data represent colonization or contamination (Broens et al., 2012). For example, the skin, nose, or tonsil could be the primary site for *S. aureus* colonization of pigs, with the other sites being contaminated from the primary site. It has been suggested that housing of pigs in isolation could give some insight into which locations are truly colonized (Broens et al., 2012). Regardless, for pigs housed in groups on commercial farms, any of these sites should be adequate for sampling of herds for epidemiological studies.

Major limitations of this study are that only 2 herds were studied, and the sample size for each sampling event was only 12 pigs. There was no credible prior data for expected prevalence, but the core assumption was that prevalence would be high in at least one anatomical site based on the general opinion that *S. aureus* is part of the normal bacterial flora of pigs. Given the high prevalence of *S. aureus* found in most anatomical sites, the small number of pigs sampled is probably a minor concern. However, as the study was conducted on only 2 commercial pig farms, the data may have limited external validity.

Tables

Table 1 – *S. aureus* prevalence in pigs by age category at pig level (at least one anatomical site culture positive in a pig)

Pig age category	***S. aureus* prevalence***	**95% Confidence interval of *S. aureus* prevalence**
Sows (adult)	89.6%	77.0, 95.7%
Preweaning piglets	87.5%	74.5, 94.4%
Nursery-age pigs	91.7%	79.5, 96.9%
Finishing-age pigs	95.8%	84.5, 99.0%

* There was no statistical difference of *S. aureus* prevalence between age groups at alpha level of 0.05.

Table 2 – Proportion of samples culture positive for *S. aureus* by age category

Pig age category	***S. aureus* prevalence***	**95% Confidence interval of *S. aureus* prevalence**
Sows (adult)	40.7% [a]	34.0, 47.7%
Preweaning piglet	38.0% [a]	30.3, 46.4%
Nursery-age pig	63.2% [b]	54.7, 71.0%
Finishing-age pig	75.2% [c]	67.3, 81.7%

* Same letters in the columns indicate no statistical difference between variables among groups at alpha level of 0.05.

Table 3 – Proportion of samples positive for *S. aureus* anatomical site

Anatomical site	***S. aureus* prevalence***	**95% Confidence interval of *S. aureus* prevalence**
Feces	42.0% [a]	34.6, 49.8%
Nose	67.9% [b]	60.4, 74.6%
Skin	62.3% [b]	54.5, 69.4%
Tonsil	61.7% [b]	54.0, 68.9%
Vagina	39.6% [a]	24.6, 56.8%

* Same letters in the columns indicate no statistical difference between variables among groups at alpha level of 0.05.

Chapter 3 –*S. aureus* prevalence in air, environmental surfaces of swine barns, pigs and humans in two swine farms in Minnesota.

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that commonly colonizes the skin and mucosal surfaces of many mammalian and avian species, including pigs. *S. aureus* is also an opportunistic pathogen that is a major cause of bacterial infections of humans, ranging from minor skin infections to fatal invasive disease. The ability of *S. aureus* to acquire genes that confer resistance to multiple drugs has further elevated its importance to public health (Cuny and Witte, 2008a). In particular, clones of *S. aureus* that are resistant to methicillin and other beta-lactam antimicrobials (MRSA) are a major clinical problem, and the discovery of MRSA in livestock populations has raised concerns about the potential importance of livestock as reservoirs of MRSA (Voss et al., 2005).

Despite being considered ubiquitous in production animal facilities (Frana, 2012), *S. aureus* ecology in livestock production farms is poorly documented. Most recent research has used selective enrichment methods to study MRSA in swine populations, rather than generic *S. aureus*. As reported in the previous chapter, *S. aureus* can be isolated from several anatomic sites of pigs. Previous studies of swine farms have reported isolation of *S. aureus* from air, environmental samples and persons having contact with pigs (Elliott et al., 1976; Frana et al., 2013; Osadebe et al., 2013). Osabede and colleagues reported *S. aureus* isolation from 30% and 22% of pigs and farm workers sampled at pig production systems in Connecticut, respectively (Osadebe et al., 2013). However, that cross-sectional study did not report *S. aureus* prevalence over time in different phases of pig production, nor the occurrence of *S. aureus* in air or environmental samples. Dust samples have been used to assess the presence of MRSA on swine farms (EFSA, 2009),

and the barn environment is a potential reservoir for *S. aureus* even after cleaning and disinfection procedures are used (Merialdi et al., 2013). Isolation of *S. aureus* in air samples from swine barns suggests this is likely an important route of exposure for people working in livestock facilities (Gibbs et al., 2006; Oppliger et al., 2012).

The objective of this study was to describe the occurrence of *S aureus* in air, environmental surfaces, pigs and farm workers of two commercial pig farms in Minnesota.

Materials and methods

Study design and source population

A longitudinal study was conducted in 2 multiple site pig production systems located in Minnesota, United States. The farms were selected by convenience based on willingness of the producers to participate and proximity. Sampling was conducted in the farrowing rooms at the breeding herd sites, and during the nursery and finishing phases at growing pig sites. Two cohorts were sampled in each system, with an interval of at least 6 months between cohorts. In both systems, piglets were weaned at 3 weeks of age and transferred to the off-site growing facilities located within 10 Km from the breeding farms. At each sampling event, samples were collected from pigs, air, and environmental surfaces of the barn, and from humans (farm workers and researchers) working in the facilities.

Animal sampling

Using sterile swabs (BBL CultureSwab[e], Liquid Stuart medium single plastic applicator, Becton, Dickinson and Co., Sparks, MD, USA) nasal, tonsil, fecal, skin (axilla) were collected from 192 pigs as described earlier in Chapter 2. Vaginal samples were also collected from the sows (total 48 samples). To sample the nares, a single swab was inserted 2 to 7 cm (according to pig size) into both nostrils and gently rotated against the mucosal epithelium. To enable collection of tonsillar samples, a speculum was used to open the mouth, and the swab was gently rotated against the tonsillar epithelium. The skin sample was obtained by rubbing a swab onto the axillary folds of pigs. The fecal sample was obtained by introducing a swab (about 5 cm) into the rectum of pigs and gently rotating the swab in the rectal contents. For vaginal sampling, a swab was gently rubbed against the vaginal epithelium using circular rotations, 5 cm into the vagina. Following sample collection the swabs were individually identified and stored on ice until processed in the laboratory.

All procedures with animals were approved by the University of Minnesota animal care and use committee (IACUC) by the protocol number 1105B99155.

Air sampling

Three liquid cyclonic collectors (Midwest MicroTek, Brookings, SD) capable of collecting 200-400 liters of air per minute were used as previously described (Dee et al., 2009) to collect air in the rooms housing the respective pig populations. The cyclonic

collectors were placed centrally in a section along the length of the barns suspended from aluminum poles positioned 80cm above from the ground and 1.50m from the sidewall. The pigs did not have direct contact with the devices.

For sample collection, the device was allowed to run for 30 min for each sampling event. Ten mL of Mueller-Hinton broth (Teknova) with 6.5% NaCl (Sigma) was used as the collection media. After collection, approximately 5 mL of media were recovered using a sterile syringe (Tyco-Healthcare, Kendall Monoject) and stored in sterile 15 mL polystyrene tubes (Sarstedt) on ice until tested. The collector was then disinfected with alkyl dimethyl benzyl ammonium chloride spray (Lysol, Reckitt Benckiser), rinsed with water, and dried with paper towels (Kim wipes, Kimberly-Clark). A total of 6 air samples were collected at each farm visit (total 72 samples).

Environmental sampling

Ten environmental samples were collected from the rooms housing the groups of animals being sampled.

Swiffer sweeper dry cloth unscented ^{TM} pads (The Procter & Gamble Company, Cincinnati, OH), were placed in a 50mL screw-top tube containing 10mL of Mueller-Hinton broth (Teknova) with 6.5% NaCl (Sigma). Each Swiffer pad was rolled and inserted into the tube and allowed to soak. This preparation was conducted in the laboratory on the day of collection. Inside the barn, the Swiffer pad was removed from the tube and wiped over surfaces of feeders, pipes and pen dividers, collecting

environmental dust and avoiding surfaces that pigs could contact directly. Each soiled Swiffer pad was replaced into the 50 mL tube and immediately stored on ice during transport to the laboratory. In the laboratory the soiled Swiffer pads were placed into a Ziploc Sandwich ® bag (S. C. Johnson & Son, Inc; Racine, WI) and the bag was squeezed. Contents from the bag were drained back into a new sterile 50 mL tube. Tubes were sealed and labeled appropriately. Latex gloves were changed between samples.

Human sampling

Nasal swabs were collected from farm workers who signed the informed consent to be sampled and University of Minnesota (UofM) personnel who were involved in sample collection. To collect their own nasal sample, each person took a sterile swab (BBL CultureSwab[e], Liquid Stuart medium single plastic applicator, Becton, Dickinson and Co., Sparks, MD, USA) unwrapped and removed the swab from the package and placed the swab into both nostrils (distal half inch of nasal epithelium) rotating 3 times using a single swab. The swabs were placed in tubes with the transport medium and immediately stored on ice until tested. All procedures with humans were approved by the University of Minnesota Institutional Review Board (IRB) by the code number 1105M00327.

Isolation and Identification of S. aureus *bacteria*

All samples were cultured in parallel by two culture procedures, described as selective (specific for culture of MRSA) and non-selective enrichment techniques (for MSSA) as described on chapter 2 with slight modifications for the environmental and air samples.

Instead of placing the swabs in a 15 ml tube (BD Falcon 15ml Conical Centrifuge tubes, Fisher Scientific) with 5 ml of Mueller-Hinton broth (Teknova) with 6.5% NaCl (Sigma), 1 mL of the liquid from the environmental or air samples was placed in the 15ml tubes (BD Falcon 15ml Conical Centrifuge tubes, Fisher Scientific Inc, Waltham, MA) with 5 ml of Mueller-Hinton broth (Teknova, Hollister, CA) containing 6.5% NaCl (Sigma-Aldrich, Saint Louis, MO) and incubated at 37°C for 24 hours. The subsequent steps for culture and identification are as described in chapter 2.

Outcomes and statistical analysis

This study describes the frequency of *S. aureus* isolation from samples from pigs, environment, air and workers of pig operations in Minnesota, United States. Additionally, to better communicate the findings, a regression model was used consolidating *S. aureus* results from each sample type. Considering the hierarchical nature of the samples, the mixed logistic regression model using Proc Glimmix from the SAS package version 9.3 (SAS Institute Inc., Cary, North Carolina), was used considering sample types (pig, environmental, air or humans) nested within production phase (farrowing, nursery or finishing-age), which was nested within cohort which was nested within farm. Thus, the sample type was considered a fixed effect in the model while production-phase, cohort and farm were considered random effects.

A pig was considered positive if *S. aureus* was isolated from one or more samples from

nose, tonsil, skin, feces or vagina.

Results

No MRSA was found on any sample from this study. All positive results reported in this study refer to isolation of MSSA. Regardless of farm and cohort, *S. aureus* was detected least frequently in air samples and most frequently from pigs and humans (Table 1). The environmental samples showed marked variation in isolation of *S. aureus* among sampling events, ranging from 0% to 80% (Table 1).

The prevalence of *S. aureus* positive samples was numerically similar between cohorts of the same farm, with the exception of environmental samples collected from at finishing-age pig phase facilities, where cohort 1 and cohort 2 had 70% and 0% positive samples, respectively.

Comparison of *S. aureus* isolation prevalence between sample sites (table 2) showed that pigs had higher prevalence than humans (p-value <0.0001), which in turn had higher prevalence than environmental samples (p-value < 0.0001). Environmental samples had higher prevalence than that of air samples (p-value 0.0006).

Discussion

The core rationale for this pilot study was that there has been no prior systematic effort to describe the occurrence of *S. aureus* in swine production systems. The vast majority of

studies have focused on MRSA strains using selective culture methods, and/or focused on a limited number of matrices. The objective was therefore to obtain preliminary descriptive data on the occurrence of *S. aureus* in pigs, people, environmental and air samples on 2 pig farms and gain some insight into the distribution of the organism in the swine farm milieu.

S. aureus was isolated most frequently from pigs and humans, and less often in the environmental and air samples (Tables 1 and 2). Although the proportions of positive samples differed significantly among these matrices, the sampling protocols varied among the matrices. Firstly, pigs were deemed positive if one or more samples from multiple anatomical sites were culture positive (Chapter 2). In contrast, only nasal swab samples were obtained from human subjects. A more appropriate comparison is between nasal swabs samples from pigs, for which the prevalence (65%, Chapter 2) was almost identical to that found in humans (68%). It has previously been observed that *S. aureus* prevalence in pig farmers is higher than the general population, and that this difference is attributable to of *S. aureus* variants that are common in pigs (Armand-Lefevre et al., 2005).

The common occurrence of *S. aureus* in environmental samples on these farms is concordant with previous findings indicating that environmental sampling can be a useful and non-invasive method for assessing *S. aureus* in swine herds (Raszyk, 1986; Friese et al., 2012). It is important to recognize swine farm environments are very diverse across farms and not homogeneous within farms, and variability in results should be expected based on choice of the location for sampling, and the nature of sampling method (e.g., area or volume of material sampled). Comparison among different studies using

environmental sampling should therefore be done with caution. Assuming the isolates obtained from environmental sampling are representative of those occurring in the respective swine population (Chapter 4), such samples will likely be most useful for determining the range of *S. aureus* variants (e.g., spa types or MRSA vs. MSSA) present in herds.

S. aureus has been previously reported in the air (Elliott et al., 1976; Gibbs et al., 2004; Friese et al., 2012; Schulz et al., 2013) of pig barns. In a recent study in Switzerland MSSA were detected in air in 30% of farms and the mean airborne concentration (DNA copy number of staphylococci) was estimated to be 35 (± 9.8) × 10^5 copy numbers per cubic meter of air (Masclaux et al., 2013). Detection of bacteria in air samples is highly influenced by methodological factors, including the equipment used for air sampling (Dungan and Leytem, 2009). Also, air quality can vary greatly within a swine facility within the course of a day and from day to day (Kim et al., 2005). In this study, air sampling was done by convenience when animal samples were being collected. The cyclonic sampler employed is a liquid impingement system that has been used extensively by our group for studies of swine viruses [Porcine reproductive and respiratory syndrome (PRRS) and swine influenza] using PCR methods (Linhares et al., 2012; Corzo et al., 2013). However, this system has not been previously evaluated for its effect on culturable organisms. The low prevalence of positive air samples found in the study, in which *S. aureus* was highly prevalent in pigs and environmental samples) may possibly be due to poor survivability of the organisms. However, no *S. aureus* were detected in two thirds of farms in Switzerland using a filtration method. Perhaps use of another air sampling device (e.g., the high volume open-faced electret filter-based air

sampler such as the SASS 3100 Dry Air Filter Sampler, Research International, Inc, Monroe, WA) which maintains integrity of the captured bacteria and collects up to 360 liters of air per minute (Wulf and Voss, 2008) could have increased the yield of *S. aureus* detection in the air. Moreover, presence of inhibitory components or competing organisms might have repressed growth of *S. aureus* in air and/or environmental samples (PETERSON et al., 1962). It is also conceivable that dust particles from antibiotic-treated feed may have inhibited *S. aureus* growth from air or environmental samples (Murphy et al., 2007).

It has been suggested that pigs play an important role in the transmission of *S. aureus*, including MRSA, to humans (Lee, 2003; Huijsdens et al., 2006). The relatively high prevalence of *S. aureus* in both humans and pigs found in this study further confirms that people working in confinement swine facilities are regularly exposed to bacterial flora of animals. However, health risks associated with these exposures remain poorly documented. It has been reported that about 40% of slaughtered pigs in the Netherlands were carriers of MRSA (Wulf and Voss, 2008). Reliable national prevalence data of MRSA in pigs are not available, but research to date suggests that the MRSA prevalence in United States pigs appear to be lower than in many European countries with major swine industries (Smith et al., 2013).

Results from chapter 2 showed no significant difference of *S. aureus* prevalence between dam and respective piglet at the farrowing barn. Therefore, for this study results from sows and piglets (farrowing phase) were then pooled for all the descriptions and comparisons reported.

S. aureus prevalence was generally consistent within sample types between different farms and cohorts, despite the relatively small sample sizes employed. The only matrix that showed high variability in frequency of *S. aureus* isolation was the environmental samples. At the facilities of the nursery phase of pig production on farm 1, the proportion of *S. aureus* positive samples shifted from 0 to 6 out of 10 samples between cohorts 1 and 2 respectively. Similarly, at the finishing-age pig phase on farm 2, it declined from 7 to 0 out of 10 samples (Table 1). However, the barns of cohort 1 and 2 were not the same for the growing phases of pig production (nursery and finishing-age pigs) and therefore barn layout, type of construction material and even climate changes between cohorts 1 and 2 might have explained the shifts of *S. aureus* isolation frequencies on environmental samples in that cohort.

As in Chapter 2, the interpretation of the data in the pilot study must consider the limitations of the design, particularly the limited sample sizes at each sampling event and the fact that only 2 systems were studied.

Tables

Table 1 – Frequency of isolation of *S. aureus* from air, environment, pig and human samples, by pig production phase and by farm and cohort.

	Farm1		Farm 2		Total
	Cohort 1	***Cohort 2***	***Cohort 1***	***Cohort 2***	
Farrowing room (breeding herd site)*					
Air	1/6	0/6	0/6	1/6	2/24
Environment	0/10	1/10	6/10	2/10	9/40
Human	1/4	2/3	8/9	5/9	16/25
Pig	22/24	21/24	18/24	24/24	85/96
Nursery phase*					
Air	1/6	2/6	0/6	2/6	5/24
Environment	0/10	6/10	5/10	2/10	13/40
Human	3/4	3/3	3/3	1/2	10/12
Pig	8/12	12/12	11/12	12/12	43/48
Finishing phase*					
Air	0/6	0/6	1/6	0/6	1/24
Environment	8/10	3/10	7/10	0/10	18/40
Human	3/3	1/3	3/3	1/4	8/13
Pig	12/12	12/12	12/12	10/12	46/48

* Table shows number of positive samples out of total number of samples collected by type at each farm visit.

Table 2 – *S. aureus* prevalence in air, environmental, human and pig samples from two commercial pig production systems located in Minnesota

Sample type	*S. aureus* prevalence*	Lower 95% confidence limit	Upper 95% confidence limit
Air	8.9%[a]	3.7%	20.0%
Environmental	31.2%[b]	18.8%	47.0%
Human	68.5%[c]	49.0%	83.0%
Pig	92.8%[d]	85.9%	96.4%

* Different letters indicate significant different prevalence between sample types at alpha level 0.05.

Chapter 4 – *Staphylococcus aureus* spa types isolated from swine, human and environmental samples from two swine farms in Minnesota, USA

Introduction

Staphylococcus aureus (*S. aureus*) is a common colonizer of both humans and pigs (Lowy, 1998; Frana, 2012). However, the role of pigs in *S. aureus* transmission to humans is yet to be determined (Cuny and Witte, 2008b). The limited information on the ecology of *S. aureus* in the pork production chain limits the ability of the swine industry to understand and communicate the risks to public health in an informed manner.

Results from the chapters 2 and 3 showed that *S. aureus* can be isolated from air, environmental samples, pigs and people on swine farms. In pigs, *S. aureus* occurs in multiple anatomic sites (nose, tonsils, skin, feces and vagina). *S. aureus* prevalence was found to be higher in pig and human samples compared to air and environmental samples. In pigs, the nose, tonsils and skin were more often positive for *S. aureus* than feces or the vagina of sows. Until recently, there has been negligible information regarding the genetic diversity of *S. aureus* found in livestock. Livestock may be reservoirs of some variants of MRSA, most notably the ST398 and ST9 lineages, which are capable of colonizing humans. The origin of livestock associated MRSA remains to be defined, and better understanding of the genetic subpopulations of *S. aureus* in swine could give insight into this question.

Several methods have been employed for subtyping *S. aureus*, including phage typing, pulse field gel electrophoresis (PFGE), multilocus sequence typing (MLST), spa typing, and SCCmec typing (Robinson and Enright, 2004; Faria et al., 2008; Monecke et al., 2011). Although PFGE is the standard method used historically in medical laboratories in many countries, sequence based methods (MLST, spa typing, SCCmec typing) are

increasingly used due to their greater reproducibility among laboratories and the availability of online databases that facilitate comparison of typing data from diverse geographic regions.

S. aureus bacteria possess the *spa* gene that codes staphylococcal protein A which binds immunoglobulins and is thought to play a central role in pathogenesis of infection (Cheng et al., 2011; Parker and Prince, 2012). The spa gene contains a region (the X-region) with a segment with a varying number of small (21 to 27 bp) repeats (Frénay et al., 1996). Based on the repeat patterns, isolates can be classified into different "spa types". To ensure consistency in genotyping there are computerized methods that automatically assign a genotype according to the pattern of such repeats using unique algorithms (Mellmann et al., 2007). A standard automated server to assign spa types is the Ridom program available at the website "http://spaserver.ridom.de" (Harmsen et al., 2003), which provides a Ridom spa type, a standard molecular typing method for *S. aureus* This system allows grouping and comparison of isolates for epidemiological analysis from local to global scales.

To investigate the diversity of *S. aureus* isolates on swine farms, all isolates from the research reported in the chapters 2 and 3 were typed using the Ridom spa server. Specific objectives were to (a) describe spa types found in each pig anatomic site (nose, skin, tonsil, fecal and vagina), (b) describe spa types isolated from each sample type (air, environment, pigs, humans), (c) compare spa types isolated from sows, their respective piglets and over time at nursery-age pigs and at finishing-age pigs and (d) compare changes in *S. aureus* diversity between different cohorts within pig production systems.

Materials and methods

Study design and source population

A longitudinal study was conducted in 2 conveniently selected independent pig production systems located in Minnesota, United States. In each production system, the breeding site was visited to collect samples from 12 lactating sows and one piglet from each sow (farrowing phase) in each cohort. Piglets in both systems were weaned at approximately 3 weeks of age. Subsequent sampling of the same birth cohorts of pigs occurred 4 and 20 weeks post weaning at "nursery" (n=12) and "finishing age" (n=12) phases of pig production, respectively. During each farm visit (farrowing phase, nursery, finishing-age) samples were also collected from farm workers (at least 2 samples per visit) and the research workers, air (n=6) and environmental surfaces (n=10). Two cohorts were sampled from each farm, with a 6 months lag period between samplings.

Isolation and Identification of S. aureus

All samples were cultured in parallel by two culture procedures, described as selective (specific for culture of MRSA) and non-selective enrichment techniques (MSSA) as described on chapter 2. Once pure cultures were obtained, and confirmed as positive for *S. aureus* the samples were stored in 0.5ml of 37% glycerol at -80°C until further genotypic characterization.

Genotypic characterization:

DNA extraction:

From the glycerol solution, each *S. aureus* isolate (n = 550) was streaked to a Columbia-CNA agar plate (Media, Prepared Plate; BD Diagnostic Systems BBL Columbia CNA Agar with 5% Sheep Blood). The plates were incubated at 37°C for 24 hours. One fresh colony was added into a PCR microplate (Genomic; 96-Well; Eppendorf; Twin.tec; PCR; Yellowr; Extra-thin polypropylene wells; Semi-skirted; Polycarbonate frame; Automation friendly, Fisher Scientific) with 19.5µl of 10mM Tris HCl (TRIS HCL, 500G, Research Products International Corp) and 0.5µl of 1 mg/ml Lysostaphin (Staphylococcus, Sigma). Then, plate was heated at 37°C for 15 minutes and was stored at -20°C for further PCR procedure.

DNA sequence analysis and Spa typing:

All *S. aureus* isolates found in this study were typed by DNA sequence analysis of the X region of the staphylococcal protein A (spa) gene as previously described (Shopsin et al., 1999) with some modifications.

The PCR amplification of the SSR region of the spa gene was achieved by the following PCR conditions: 1.0 µl of the DNA of the samples, 9.5 µl of nuclease free water, 1.0 µl of 1095F forward PCR primer at 10µM, 1.0 µl of 1517R reverse PCR primer at 10µM, and 12.5µl of HotStart-IT FideliTaq PCR Master Mix (2X) (USB, http://www.affymetrix.com) were added in 0.2 ml PCR tubes. A negative control (nuclease free water) and a positive control were included. Tubes were capped and placed in a GeneAmp PCR System 9600 Thermocycler. Thermal cycling parameters included 30 cycles of 2 minutes at 95°C, 30 seconds at 94°C, 30 seconds at 55°C; 1 minute at 72°C,

and a final extension at 72°C for 5 minutes. Completed reaction mixtures were stored at −20°C.

The amplicons were sequenced using regular Sanger sequencing at the Biomedical Genomics Center (BMGC), University of Minnesota (http://www.bmgc.umn.edu/facilities/sequencing/services/sequencing/home.html).

DNA sequences of the spa genes from the BMGC were submitted to the eGenomics program (www.egenomics.com) and Ridom SpaServer (www.spaserver.ridom.de), which use computerized algorithms to analyze the variability of the spa gene X region and assign spa types. The eGenomics spa types are reported using a numerical system (e.g., spa type 539) while Ridom spa types are reported using a numerical system preceded by a 't' (e.g., spa t034).

Outcomes

This was a descriptive study of the frequencies of Ridom spa types (hereafter termed "spa type") of *S. aureus* isolated from pigs, humans, air and environmental samples collected at pig farms.

The Ridom spa types found in this study were used as keywords (e.g. "t337") to search the PubMed database (http://www.ncbi.nlm.nih.gov) aiming to identify published manuscripts reporting each corresponding spa type. When accessible, the full manuscripts were examined to determine the origin of the isolate (human, pigs, other) for each particular spa type. When no publications matched results, the spa type was considered as

an uncited spa type.

For isolates that had non-sequenceable DNA, "NS" (non-sequenceable) was reported. Figures were generated using Proc SGPanel of SAS software version 9.3.

The isolates that had sequences not recognized by the Ridom and/or eGenenomics server were denominated "new spa types".

Subsequent to completion of this work, selected isolates of the spa types found in the study were analyzed by multilocus sequence typing to establish their MLST lineages (Jisun Sun, unpublished data, 2013), and these results are included.

Results

All *S. aureus* isolates reported in this study were methicillin-sensitive *S. aureus* (MSSA) based on lack of growth of isolates on MRSA selective plates. There were 8 isolates (all from pigs) that were not sequenceable (NS) and therefore could not be characterized by spa typing.

Overall, 15 Ridom spa types were identified among the 513 isolates from the two farms studied. One spa type identified on 26 samples (air, environment, pigs and humans), with the Ridom motif sequence "T1-J1-M1-B1-M1-D1-M1-G1-M1-K1" was initially not typed in the Ridom system. This spa typed was eGenomics spa type 2 and based on the motif was determined to be Ridom spa type t002. Another spa type with the Ridom motif sequence "U1-J1-J1-A1-G1-J1-A1-A1-B1" was not typable with either the Ridom or

eGenomics systems.

A number of spa types (N = 5) were identified by the Ridom server but could not be identified in previous publications by the search process employed. For the purpose of this study they are called “uncited spa types”. The uncited spa types were t216 and t4106 from human samples; t3446 and t7331 from both pig and human samples; and spa type t2462 from pig samples.

S. aureus diversity by pig anatomic site

The most frequent spa types found in pigs were t034 (35.8% of spa types found in pigs), t337 (27.9%) and t7331 (13%).

Isolates obtained from pigs (Table 1) originated from several anatomic sites: nose, skin, tonsil, feces, or vagina (sows only). Several spa types were isolated from all pig anatomic sites: t034, t2462, t337 and t3446. Type t7331 was found in all anatomic sampled sites except vagina, and spa type t571 was isolated in nose, skin and tonsil samples, but not feces or vagina. The spa type t1255 was found only in nose and skin and t526 was found only in tonsils. There was little evidence to suggest that particular spa types were associated with specific anatomic sites.

*S. aureus diversity by sample type (*pig, air, environment and human)

All the spa types found on air or environmental samples were also found in humans and/or pigs (Table 1, Figure 1). A broader array of spa types were found in pigs and

humans than in air and environmental samples, but likely reflects the greater number of isolates obtained from the animal and human samples.

The spa types that were found in both pigs and humans included the most prevalent spa types in pigs (t034, t337, t7331, t3446, t002 and the new spa type). Several spa types (t5883, t216, t4106) were found only in humans or in pigs (t1255, t2462, t571 and t526, new spa type).

S. aureus diversity in pigs by age category and cohort

The pig age categories sampled were sows with their respective piglets and (following the same birth cohorts) nursery-age pigs (4-10 weeks) and at finishing pigs (11 – 28 weeks) (Table 1, Figures 1 and 2). The first and second cohorts in each system were separated by a period of 4 to 6 months.

Results from pigs sampled in cohorts 1 and 2 at farm 1 (Figure 2) showed pigs may be simultaneously colonized by multiple spa types. Spa types found sows and piglets in each cohort generally corresponded closely at the group level, although spa types found in sows were not necessarily those found in their respective piglets.

In one cohort (farm 1, cohort 1), the spa type t7331 was not detected at the breeding site (where sows and pre-weaning piglets were housed) but was the predominant spa type in nursery and finishing age pigs of that cohort. However, spa type t7331 was not detected in any pigs of the second cohort of this system (Farm 1, cohort 2) in which spa type t034 was the predominant spa type among the isolates from growing pigs (nursery and finishing age pigs).

Data of pig spa types at farm 2 (Figure 2) similarly showed that multiple spa types can be occur in individual pigs simultaneously, and that spa types found in sows were not always the same as those found in their respective piglets. Also, at farm 2 several spa types (t034, t1255, new) were isolated from growing pigs despite not being found at the breeding site, but the spa type t337 was commonly found in all age groups of both cohorts.

Figure 2 indicates that the patterns of spa types found in pigs might or might not change over time according to pig age group and/or pig cohort within a production system.

The MLST types of selected isolates from the predominant spa types found in the study are listed in Table 2. All spa types tested grouped within 3 MLST sequence types of ST398, ST9, and ST 5 (Table 2)

Discussion

It is typical that organisms considered to be 'normal flora' of a species (e.g., *E, coli* in most species) generally display considerable genetic diversity within a host species or even an individual animal (Leimbach et al., 2013). A peculiar feature of the early research on MRSA in pigs was that the organisms detected (ST398 MRSA) were apparently closely related and often described as to be a novel clone (van Loo et al., 2007). Limited diversity of an organism within a species would typically imply a relatively recent evolutionary relationship. Some commensal organisms vary quantitatively (prevalence) and qualitatively (strains of organisms) as animals age. A

study of the nasal tonsillar flora of pigs before and after weaning observed changes in the composition of the flora, and reported that *S. aureus* and most lactobacilli became more prevalent after weaning (Baele et al., 2001). However, no subtyping was conducted in that study. One earlier study (Armand-Lefevre et al., 2005), and a recent study published after the completion of our field work (Oppliger et al., 2012) reported that diverse subtypes (MLST types and spa types respectively) were found in pigs and people in contact with them. However, those studies were cross-sectional and did not describe patterns of colonization in different age groups of pigs.

For Farm 1, decline in diversity of spa types was marked in both cohorts from the breeding to the growing phases, but the predominant spa types found in the growing pig phases differed between cohorts (t034 and t7331). No similar pattern was evident in Farm 2, suggesting that it is unlikely to be a normal feature of weaning or aging. However, it appears that some selective pressures favored the predominance of individual spa types in the growing pig populations of Farm 1. At both breeding sites, multiple spa types were present on both farms and appeared not to differ greatly between cohorts. Overall, spa types from piglets at a site were similar to those from sows. Studies designed to characterize *S. aureus* spa types in swine populations should consider the observations that spa type profiles are likely to differ among age groups of pigs, and to vary over time.

It is notable that in both farms multiple spa types were isolated from individual pigs simultaneously. As pigs were housed in groups, it is impossible to discriminate between true colonization of a site versus transient contamination from another source (pig or environment). It is usual for multiple variants of some commensals to coexist in an

animal (e.g. *E. coli*). Isolation of individual pigs would be required to determine whether multiple variants truly colonized individual pigs. While biologically interesting, this question has little relevance as pigs are routinely raised in groups, and in this setting multiple variants of *S. aureus* can be expected to occur in individual animals.

In agreement with numerous previous reports, this study found that the same spa types can be found in pigs and humans present in the swine farm environment (Khanna et al., 2008; Smith et al., 2009). Most human sampling has been based on nasal and oropharyngeal swabs, and *S. aureus* can be detected in the air of swine barns. Questions remain whether culture positive results from the upper respiratory tract of people represent colonization or simply transient contamination. A recent longitudinal study of swine veterinarians in Holland suggests that true colonization may occur in minority of individuals and that transient contamination is more frequent (Verkade et al., 2013). However, similar to another study (Sergio et al., 2007) we observed some spa types were detected exclusively in humans or pigs which could reflect sampling error (particularly for the small number of human subjects) or the existence of human variants that are not readily transmitted to swine. Arguably, the ability to detect human variants being transmitted to swine under field conditions is probably negligible given the high prevalence of colonization of pigs with swine adapted lineages.

Our observations confirm that considerable diversity of *S. aureus* spa types occurs within pig farms, and even within individual animals. Furthermore, the data indicate that predominant spa types may differ between farms, between cohorts within farms, and within a cohort over time. It is important to note that the study was conducted on only 2 farms, 2 cohorts per farm, and that the number of samples per sampling event was

modest. Therefore, the data may have limited external validity, and sampling error rather than biological variation may be responsible for some of the variability observed, particularly in spa type distributions. For example, presence of a particular spa type in a cohort at one phase of production but not others may be explained simply by the small number of animals sampled at any stage. On the other hand, the large number of isolates evaluated on these farms overall greatly exceeds that in earlier studies, and likely provides reasonably reliable identification of the predominant spa types on the farms overall. For comparison, Armand-Lefevre and colleagues (Armand-Lefevre et al., 2005) sampled only 14 swine *S. aureus* isolates from laboratory submissions to veterinary laboratories across France. Oppliger et al (2012) conducted a broader but more superficial study, sampling a mean of 8.8 pigs per farm across 41 farms (Oppliger et al., 2012). Osadebe et al (2013) sampled up to 30 pigs on 51 farms across all age groups (Osadebe et al., 2013). A remarkable and important observation is that the three MLST types containing all the swine spa types tested in this study correspond closely with those reported by both Oppliger et al (2012) and Armand- Lefevre et al (2005), and particularly the predominance of ST9 and ST398. This is significant for 2 reasons. Firstly the remarkable similarity, despite different study designs and considerable temporo-spatial separation of the study populations, suggests that these lineages may be widely distributed among commercial swine populations across the world. However, data from one study in Senegal indicate that this observation is not universal among swine populations (Fall et al., 2012). Secondly, while ST398 is the predominant lineage of livestock associated MRSA in Europe, ST9 appears predominant in most Asian countries studied, and ST5 MRSA have been repeatedly isolated from pigs in North America

(Khanna et al., 2008; Frana, 2012). It is reasonable to postulate that the apparent emergence of livestock associated MRSA may be the result of acquisition of the mecA gene by several *S. aureus* lineages that have been adapted to swine over the long term, rather than recent introduction of novel variants into swine populations.

Tables and figures

Table 1 – Number (row percentage) of Ridom spa types identified by sample type across all farms and cohorts.

Sample	Ridom spa type														
	NS*	**New spa type**	**t034**	**t1255**	**t216**	**t2462**	**t337**	**t3446**	**t4106**	**t526**	**t571**	**t5883**	**t7331**	**t002**	**Total**
Air	0	0	3 (37.5%)	0	0	1 (12.5%)	1 (12.5%)	1 (12.5%)	0	0	0	0	1 (12.5%)	1 (12.5%)	8
Environmental	0	0	18 (40.0%)	0	0	0	16 (35.6%)	1 (2.2%)	0	0	0	0	8 (17.8%)	2 (4.4%)	45
Human	0	0	12 (35.3%)	0	2 (5.9%)	0	7 (20.6%)	6 (17.7%)	1 (2.9%)	0	0	2 (5.9%)	2 (5.9%)	2 (5.9%)	34
Pig	8 (1.8%)	1 (0.2%)	163 (35.8%)	3 (0.7%)	0	33 (7.3%)	127 (27.9%)	31 (6.8%)	0	1 (0.2%)	8 (1.8%)	0	59 (13.0%)	21 (4.6%)	455
Fecal	1 (1.2%)	1 (1.2%)	28 (34.2%)	0	0	2 (2.4%)	23 (28.1%)	6 (7.3%)	0	0	0	0	17 (20.7%)	4 (4.9%)	*82*
Nose	2 (1.6%)	0	48 (37.8%)	2 (1.6%)	0	14 (11.0%)	27 (21.3%)	8 (6.3%)	0	0	3 (2.4%)	0	14 (11.0%)	9 (7.1%)	*127*
Skin	1 (0.9%)	0	43 (36.8%)	1 (0.9%)	0	8 (6.8%)	34 (29.1%)	8 (6.8%)	0	0	2 (1.7%)	0	17 (14.5%)	3 (2.6%)	*117*
Tonsil	4 (3.5%)	0	42 (36.2%)	0	0	7 (6.0%)	35 (30.2%)	8 (6.9%)	0	1 (0.9%)	3 (2.6%)	0	11 (9.5%)	5 (4.3%)	*116*
Vagina	0	0	2 (15.4%)	0	0	2 (15.4%)	8 (61.5%)	1 (7.7%)	0	0	0	0	0	0	13
Total	**8 (1.5%)**	**1 (0.2%)**	**196 (36.2%)**	**3 (0.6%)**	**2 (0.4%)**	**34 (6.3%)**	**151 (27.9%)**	**39 (7.2%)**	**1 (0.2%)**	**1 (0.2%)**	**8 (1.5%)**	**2 (0.4%)**	**70 (12.6%)**	**26 (4.8%)**	**542**

*NS: Non sequenceable.

Table 2 – MLST types of isolates of the most common spa types found in pigs*

MLST type	Spa types
ST 398	t034, t571, t1255, t5883
ST 9	t337, t2462, t3446, t7331
ST 5	t002

* There were 8 isolates that were non sequenceable – those isolates were not submitted to MLST testing.

Figure 1 – Occurrence of *S. aureus spa* types by sample type by farm and cohort.

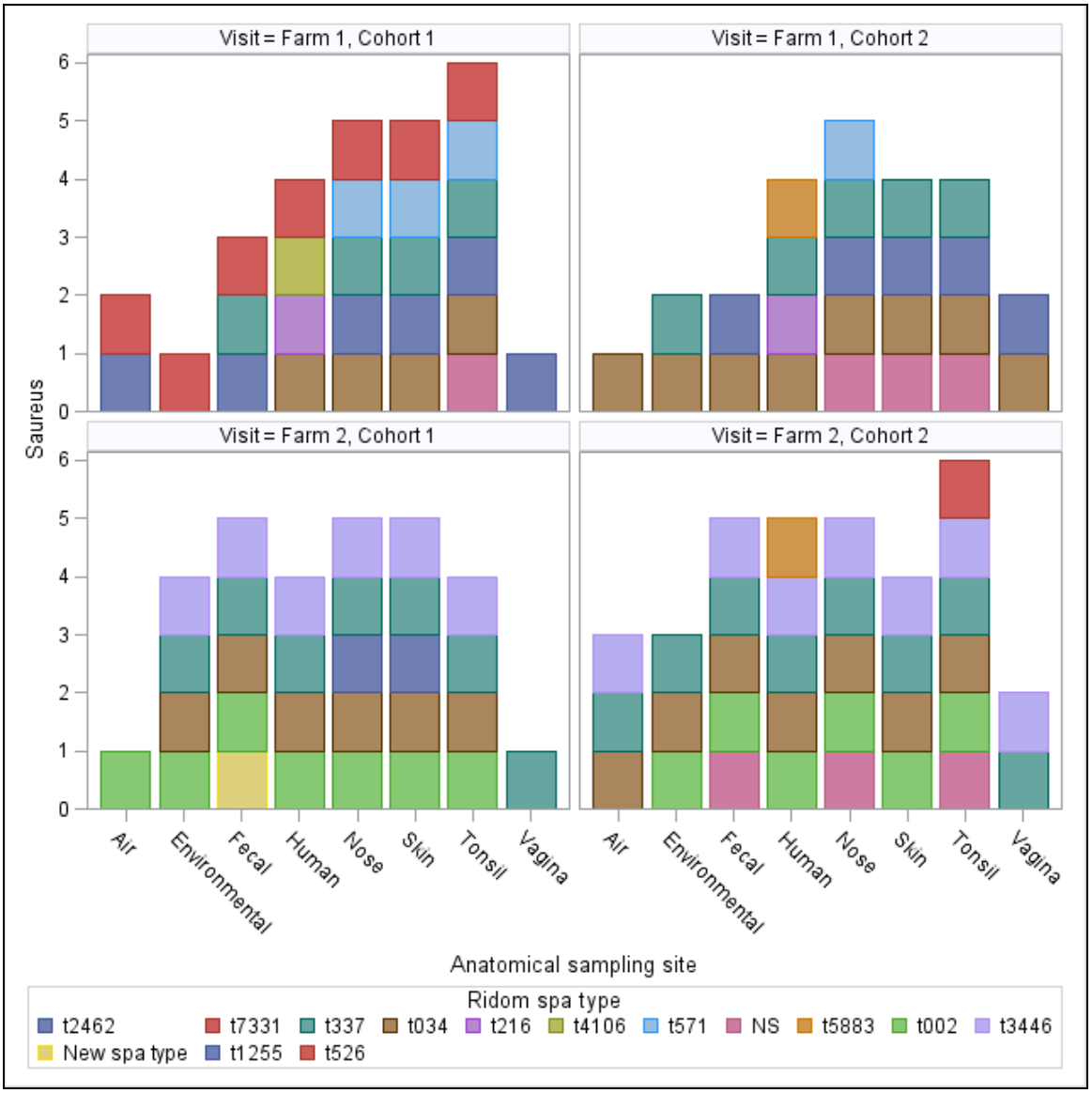
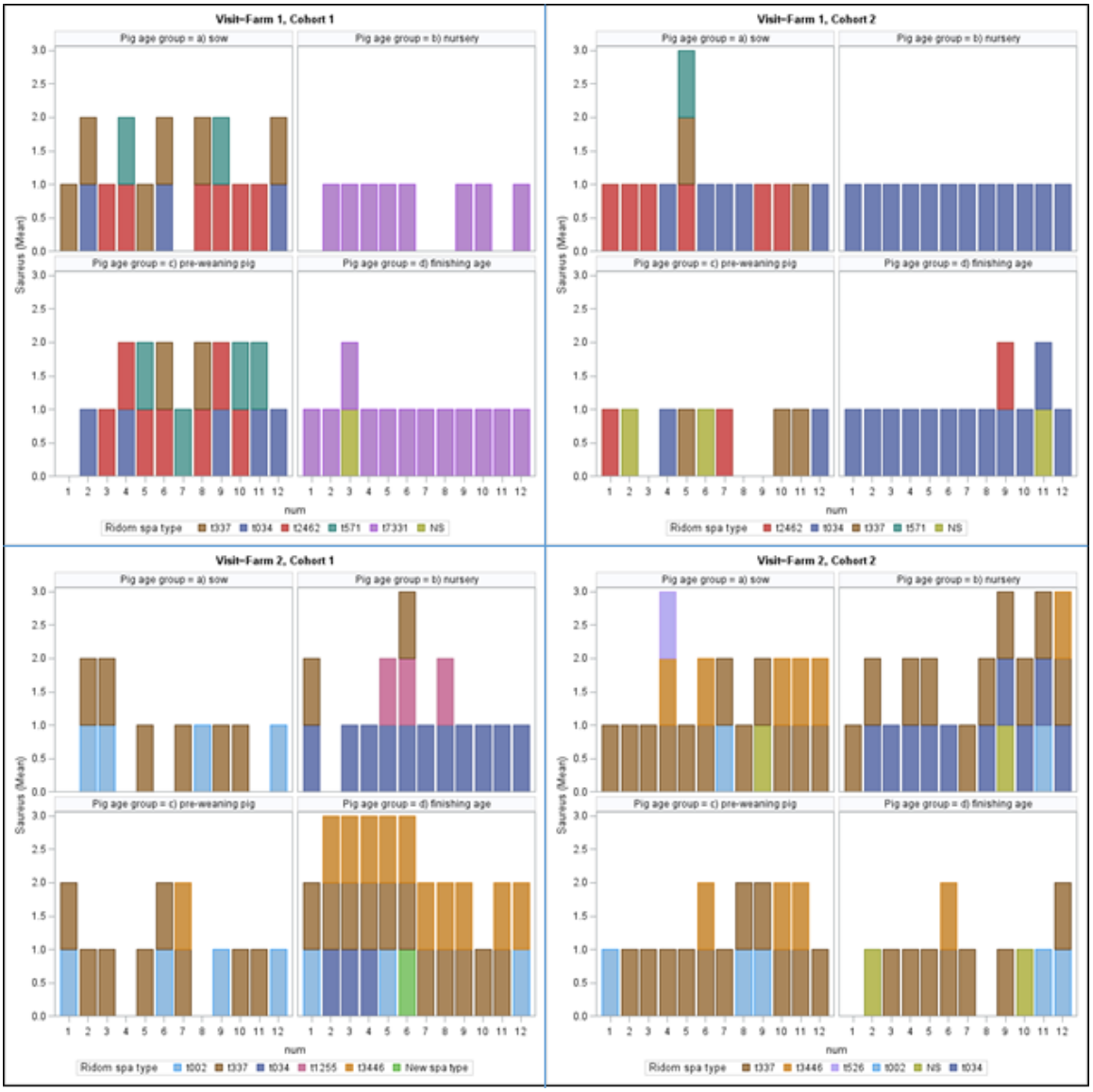


Figure 2 – Occurrence of *S. aureus* spa types by pig age category, per farm and cohort



Discussion

This thesis had the overall objective to obtain data on the ecology of *S. aureus* in pigs, people, environmental and air samples on pig farms and some insight into the distribution of the organism in the swine farm milieu.

Chapter 1 provided a concise literature review on the ecology of *S. aureus* in pig farms, reviewed basic *S. aureus* diagnostics and genotyping, and reviewed published information on transmission of *S. aureus* between pigs and humans.

All *S. aureus* found in samples on the 2 farms studied were methicillin sensitive *S. aureus* (MSSA), not MRSA). Results from chapter 2 supported the contention that *S. aureus* constitutes part of the normal bacterial flora of pigs. Approximately 90% of pigs sampled in all age groups were culture positive for *S. aureus* in at least one anatomical site. Also, the data indicated that the nose, tonsils and skin were the anatomical sites most likely to be culture positive for *S. aureus* in pigs. However, *S. aureus* was also prevalent in swine feces and in vaginal swabs of sows.

The data presented in Chapter 3 showed that *S. aureus* was detected least frequently in air samples and most frequently from pigs and from humans. The environmental samples showed marked variation in isolation of *S. aureus* among sampling events, ranging from 0% to 80%. Overall, comparison of *S. aureus* isolation prevalence between sample sites showed that pigs had higher prevalence than humans, which in turn had higher prevalence than environmental samples. Higher prevalence was found in environmental samples than in air samples, although the procedures used for sampling these matrices were very different.

Until recently, there has been negligible information available regarding the genetic diversity of *S. aureus* found in livestock. Livestock may be reservoirs of some variants of MRSA, most notably the ST398 and ST9 lineages, that are capable of colonizing humans. The data presented in chapter 4 showed that the ecology of S. aureus is complex, as 15 Ridom spa types were identified in isolates from the 2 farms. One of these was a new type note previously recognized. Given that only 12 pigs were sampled in each group, it is likely that the diversity of spa types on these farms is indeed greater than observed. Some spa types appeared to be farm and/or cohort specific, although these differences could be attributable to sampling error, particularly at the cohort level. Also, it was found that individual pigs can harbor multiple spa types and spa types from suckling piglets at a site were generally similar to those isolated from their dams. Moreover, some spa types were found only in pigs or humans while others were found in both. However, all spa types found on air or environmental samples were also found in humans and/or pigs.

Overall, results from this thesis contribute to a better understanding of the ecology of *S. aureus in* pig farm environment including pigs, air, dust and people. Knowledge generated from this study also allows better understanding of *S. aureus* occurrence in livestock and the genetic relationship between isolates found in different anatomic pig sites, dust, air and humans, which is crucial to take informed decisions on sampling strategies or monitoring strategies of *S. aureus* in such environments.

References

Aires-de-Sousa, M., Boye, K., de Lencastre, H., Deplano, A., Enright, M.C., Etienne, J., Friedrich, A., Harmsen, D., Holmes, A., Huijsdens, X.W., Kearns, A.M., Mellmann, A., Meugnier, H., Rasheed, J.K., Spalburg, E., Strommenger, B., Struelens, M.J., Tenover, F.C., Thomas, J., Vogel, U., Westh, H., Xu, J., Witte, W., 2006. High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. J Clin Microbiol 44, 619-621.

Akatov, A.K., Samsonova, T.M., Khatenever, M.L., Kushko, I.V., Shiriaeva, V.I., 1983. [Classification and biological characteristics of coagulase-positive Staphylococci isolated from animals]. Zh Mikrobiol Epidemiol Immunobiol, 29-33.

Alt, K., Fetsch, A., Schroeter, A., Guerra, B., Hammerl, J.A., Hertwig, S., Senkov, N., Geinets, A., Mueller-Graf, C., Braeunig, J., Kaesbohrer, A., Appel, B., Hensel, A., Tenhagen, B.A., 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet Res 7, 69.

Andresen, L.O., Ahrens, P., Daugaard, L., Bille-Hansen, V., 2005. Exudative epidermitis in pigs caused by toxigenic Staphylococcus chromogenes. Vet Microbiol 105, 291-300.

Armand-Lefevre, L., Ruimy, R., Andremont, A., 2005. Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11, 711-714.

Baele, M., Chiers, K., Devriese, L.A., Smith, H.E., Wisselink, H.J., Vaneechoutte, M., Haesebrouck, F., 2001. The gram-positive tonsillar and nasal flora of piglets before and after weaning. J Appl Microbiol 91, 997-1003.

Battisti, A., Franco, A., Merialdi, G., Hasman, H., Iurescia, M., Lorenzetti, R., Feltrin, F., Zini, M., Aarestrup, F.M., 2010. Heterogeneity among methicillin-resistant Staphylococcus aureus from Italian pig finishing holdings. Vet Microbiol 142, 361-366.

Berger-Bächi, B., Rohrer, S., 2002. Factors influencing methicillin resistance in staphylococci. Arch Microbiol 178, 165-171.

Broens, E.M., Espinosa-Gongora, C., Graat, E.A., Vendrig, N., Van Der Wolf, P.J., Guardabassi, L., Butaye, P., Nielsen, J.P., De Jong, M.C., Van De Giessen, A.W., 2012. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res 8, 58.

Broens, E.M., Graat, E.A., Engel, B., van Oosterom, R.A., van de Giessen, A.W., van der Wolf, P.J., 2011a. Comparison of sampling methods used for MRSA-classification of herds with breeding pigs. Vet Microbiol 147, 440-444.

Broens, E.M., Graat, E.A., Van der Wolf, P.J., Van de Giessen, A.W., De Jong, M.C., 2011b. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev Vet Med 102, 41-49.

Cheng, A.G., DeDent, A.C., Schneewind, O., Missiakas, D., 2011. A play in four acts: Staphylococcus aureus abscess formation. Trends Microbiol 19, 225-232.

Chua, K.Y., Howden, B.P., Jiang, J.H., Stinear, T., Peleg, A.Y., 2013. Population genetics and the evolution of virulence in Staphylococcus aureus. Infect Genet Evol.

Corzo, C.A., Romagosa, A., Dee, S.A., Gramer, M.R., Morrison, R.B., Torremorell, M., 2013. Relationship between airborne detection of influenza A virus and the number of infected pigs. Vet J 196, 171-175.

Cowan, S.T., Steel, K.J., 1965. Manual identification of medical bacteria. Cambridge University Press Cambridge.

Crombé, F., Argudín, M.A., Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2013. Transmission Dynamics of Methicillin-Resistant Staphylococcus aureus in Pigs. Front Microbiol 4, 57.

Crombé, F., Willems, G., Dispas, M., Hallin, M., Denis, O., Suetens, C., Gordts, B., Struelens, M., Butaye, P., 2012. Prevalence and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus among pigs in Belgium. Microb Drug Resist 18, 125-131.

Cui, S., Li, J., Hu, C., Jin, S., Li, F., Guo, Y., Ran, L., Ma, Y., 2009. Isolation and characterization of methicillin-resistant Staphylococcus aureus from swine and workers in China. J Antimicrob Chemother 64, 680-683.

Cuny, C., Witte, W., 2008a. Importance of the spread of Methicillin-resistant Staphylococcus aureus (MRSA) in fattened pigs for humans? MMW Fortschr Med 150 Suppl 2, 65-67.

Cuny, C., Witte, W., 2008b. [Importance of the spread of Methicillin-resistant Staphylococcus aureus (MRSA) in fattened pigs for humans?]. MMW Fortschr Med 150 Suppl 2, 65-67.

Davies, P., 2012. Livestock associated MRSA: What are the risks to human health? In, Allen D. Leman Swine Conference, Saint Paul, 153-158.

Dee, S., Otake, S., Oliveira, S., Deen, J., 2009. Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae. Veterinary research 40, 39.

Diep, B.A., Otto, M., 2008. The role of virulence determinants in community-associated MRSA pathogenesis. Trends Microbiol 16, 361-369.

Dimitracopoulos, G., Kalkani-Boussiakou, H., Papavassiliou, J., 1977. Animal fecal carriership and biotypes of Staphylococcus aureus. Appl Environ Microbiol 34, 461-464.

Dungan, R.S., Leytem, A.B., 2009. Airborne endotoxin concentrations at a large open-lot dairy in southern idaho. J Environ Qual 38, 1919-1923.

EFSA, 2009. Scientific Opinion of the Panel on Biological Hazards. Assessment of the Public Health significance of meticillin resistant Staphylococcus aureus (MRSA) in animals and food. The EFSA Journal, 1-73.

Elliott, L.F., McCalla, T.M., Deshazer, J.A., 1976. Bacteria in the air of housed Swine units. Appl Environ Microbiol 32, 270-273.

Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann, H., Spratt, B.G., 2002. The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). Proc Natl Acad Sci U S A 99, 7687-7692.

ERIKSEN, K.R., ERICHSEN, I., 1963. [CLINICAL OCCURRENCE OF METHICILLIN-RESISTANT STRAINS OF STAPHYLOCOCCUS AUREUS]. Ugeskr Laeger 125, 1234-1240.

Fall, C., Seck, A., Richard, V., Ndour, M., Sembene, M., Laurent, F., Breurec, S., 2012. Epidemiology of Staphylococcus aureus in pigs and farmers in the largest farm in Dakar, Senegal. Foodborne Pathog Dis 9, 962-965.

Faria, N.A., Carrico, J.A., Oliveira, D.C., Ramirez, M., de Lencastre, H., 2008. Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains. J Clin Microbiol 46, 136-144.

Frana, T.S., 2012. Staphylococcosis. In: Zimmerman, J., Karriker, L., Ramirez, A., Schwartz, K., Stevenson, G. (Eds.), Diseases of Swine. John Wiley & Sons, Inc, Ames, IA, 1008.

Frana, T.S., Beahm, A.R., Hanson, B.M., Kinyon, J.M., Layman, L.L., Karriker, L.A., Ramirez, A., Smith, T.C., 2013. Isolation and characterization of methicillin-resistant Staphylococcus aureus from pork farms and visiting veterinary students. PLoS One 8, e53738.

Friese, A., Schulz, J., Hoehle, L., Fetsch, A., Tenhagen, B.A., Hartung, J., Roesler, U., 2012. Occurrence of MRSA in air and housing environment of pig barns. Vet Microbiol 158, 129-135.

Friese, A., Schulz, J., Laube, H., von Salviati, C., Hartung, J., Roesler, U., 2013. Faecal occurrence and emissions of livestock-associated methicillin-resistant Staphylococcus aureus (laMRSA) and ESbl/AmpC-producing E. coli from animal farms in Germany. Berl Munch Tierarztl Wochenschr 126, 175-180.

Frénay, H.M., Bunschoten, A.E., Schouls, L.M., van Leeuwen, W.J., Vandenbroucke-Grauls, C.M., Verhoef, J., Mooi, F.R., 1996. Molecular typing of methicillin-resistant Staphylococcus aureus on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect Dis 15, 60-64.

Gibbs, S.G., Green, C.F., Tarwater, P.M., Mota, L.C., Mena, K.D., Scarpino, P.V., 2006. Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. Environ Health Perspect 114, 1032-1037.

Gibbs, S.G., Green, C.F., Tarwater, P.M., Scarpino, P.V., 2004. Airborne antibiotic resistant and nonresistant bacteria and fungi recovered from two swine herd confined animal feeding operations. J Occup Environ Hyg 1, 699-706.

Grumann, D., Nübel, U., Bröker, B.M., 2013. Staphylococcus aureus toxins - Their functions and genetics. Infect Genet Evol.

Guardabassi, L., O'Donoghue, M., Moodley, A., Ho, J., Boost, M., 2009. Novel lineage of methicillin-resistant Staphylococcus aureus, Hong Kong. Emerg Infect Dis 15, 1998-2000.

Harmsen, D., Claus, H., Witte, W., Rothgänger, J., Turnwald, D., Vogel, U., 2003. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 41, 5442-5448.

Hawken, P., Weese, J.S., Friendship, R., Warriner, K., 2013. Longitudinal Study of Clostridium difficile and Methicillin-Resistant Staphylococcus aureus Associated with Pigs from Weaning through to the End of Processing. J Food Prot 76, 624-630.

Horgan, M., Abbott, Y., Lawlor, P.G., Rossney, A., Coffey, A., Fitzgerald, G.F., McAuliffe, O., Paul Ross, R., 2011. A study of the prevalence of methicillin-resistant Staphylococcus aureus in pigs and in personnel involved in the pig industry in Ireland. Vet J 190, 255-259.

Huijsdens, X.W., van Dijke, B.J., Spalburg, E., van Santen-Verheuvel, M.G., Heck, M.E., Pluister, G.N., Voss, A., Wannet, W.J., de Neeling, A.J., 2006. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob 5, 26.

Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H., Hiramatsu, K., 2004. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother 48, 2637-2651.

Katayama, Y., Ito, T., Hiramatsu, K., 2000. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother 44, 1549-1555.

Khanna, T., Friendship, R., Dewey, C., Weese, J.S., 2008. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet Microbiol 128, 298-303.

Kim, K.Y., Ko, H.J., Lee, K.J., Park, J.B., Kim, C.N., 2005. Temporal and spatial distributions of aerial contaminants in an enclosed pig building in winter. Environ Res 99, 150-157.

Kloos, W.E., 1980. Natural populations of the genus Staphylococcus. Annu Rev Microbiol 34, 559-592.

Köck, R., Siam, K., Al-Malat, S., Christmann, J., Schaumburg, F., Becker, K., Friedrich, A.W., 2011. Characteristics of hospital patients colonized with livestock-associated meticillin-resistant Staphylococcus aureus (MRSA) CC398 versus other MRSA clones. J Hosp Infect 79, 292-296.

Lee, J.H., 2003. Methicillin (Oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 69, 6489-6494.

Leimbach, A., Hacker, J., Dobrindt, U., 2013. E. coli as an all-rounder: the thin line between commensalism and pathogenicity. Curr Top Microbiol Immunol 358, 3-32.

Leonard, F.C., Markey, B.K., 2008. Meticillin-resistant Staphylococcus aureus in animals: a review. Vet J 175, 27-36.

Linhares, D.C., Cano, J.P., Wetzell, T., Nerem, J., Torremorell, M., Dee, S.A., 2012. Effect of modified-live porcine reproductive and respiratory syndrome virus (PRRSv) vaccine on the shedding of wild-type virus from an infected population of growing pigs. Vaccine 30, 407-413.

Lowy, F.D., 1998. Staphylococcus aureus infections. N Engl J Med 339, 520-532.

Lozano, C., Aspiroz, C., Charlez, L., Gómez-Sanz, E., Toledo, M., Zarazaga, M., Torres, C., 2011. Skin lesion by methicillin-resistant Staphylococcus aureus ST398-t1451

in a Spanish pig farmer: possible transmission from animals to humans. Vector Borne Zoonotic Dis 11, 605-607.
Macdonald, A., Smith, A., 1984. Classics in infectious diseases. "On abscesses". Alexander Ogston (1844-1929). Rev Infect Dis 6, 122-128.
Malachowa, N., Sabat, A., Gniadkowski, M., Krzyszton-Russjan, J., Empel, J., Miedzobrodzki, J., Kosowska-Shick, K., Appelbaum, P.C., Hryniewicz, W., 2005. Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, spa typing, and multilocus sequence typing for clonal characterization of Staphylococcus aureus isolates. J Clin Microbiol 43, 3095-3100.
Masclaux, F.G., Sakwinska, O., Charrière, N., Semaani, E., Oppliger, A., 2013. Concentration of Airborne Staphylococcus aureus (MRSA and MSSA), Total Bacteria, and Endotoxins in Pig Farms. Ann Occup Hyg 57, 550-557.
Meemken, D., Cuny, C., Witte, W., Eichler, U., Staudt, R., Blaha, T., 2008. [Occurrence of MRSA in pigs and in humans involved in pig production--preliminary results of a study in the northwest of Germany]. Dtsch Tierarztl Wochenschr 115, 132-139.
Mellmann, A., Weniger, T., Berssenbrügge, C., Rothgänger, J., Sammeth, M., Stoye, J., Harmsen, D., 2007. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms. BMC Microbiol 7, 98.
Merialdi, G., Galletti, E., Guazzetti, S., Rosignoli, C., Alborali, G., Battisti, A., Franco, A., Bonilauri, P., Rugna, G., Martelli, P., 2013. Environmental methicillin-resistant Staphylococcus aureus contamination in pig herds in relation to the productive phase and application of cleaning and disinfection. Res Vet Sci 94, 425-427.
Monecke, S., Coombs, G., Shore, A.C., Coleman, D.C., Akpaka, P., Borg, M., Chow, H., Ip, M., Jatzwauk, L., Jonas, D., Kadlec, K., Kearns, A., Laurent, F., O'Brien, F.G., Pearson, J., Ruppelt, A., Schwarz, S., Scicluna, E., Slickers, P., Tan, H.L., Weber, S., Ehricht, R., 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PLoS One 6, e17936.
Morgan, M., 2008. Methicillin-resistant Staphylococcus aureus and animals: zoonosis or humanosis? J Antimicrob Chemother 62, 1181-1187.
Mulvey, M.R., Chui, L., Ismail, J., Louie, L., Murphy, C., Chang, N., Alfa, M., Methods, C.C.f.t.S.o.M., 2001. Development of a Canadian standardized protocol for subtyping methicillin-resistant Staphylococcus aureus using pulsed-field gel electrophoresis. J Clin Microbiol 39, 3481-3485.
Murphy, M.W., Sanderson, W.T., Vargo, J.D., 2007. Airborne antibiotic concentrations in a swine feeding operation. J Agric Saf Health 13, 357-366.
Nathaus, R., Blaha, T., Tegeler, R., Meemken, D., 2010. [Intra-herd prevalence and colonisation dynamics of Methicillin-resistant Staphylococcus aureus (MRSA) in two pig breeding herds]. Berl Munch Tierarztl Wochenschr 123, 221-228.

Neela, V., Mohd Zafrul, A., Mariana, N.S., van Belkum, A., Liew, Y.K., Rad, E.G., 2009. Prevalence of ST9 methicillin-resistant Staphylococcus aureus among pigs and pig handlers in Malaysia. J Clin Microbiol 47, 4138-4140.
Oppliger, A., Moreillon, P., Charrière, N., Giddey, M., Morisset, D., Sakwinska, O., 2012. Antimicrobial resistance of Staphylococcus aureus strains acquired by pig farmers from pigs. Appl Environ Microbiol 78, 8010-8014.
Osadebe, L.U., Hanson, B., Smith, T.C., Heimer, R., 2013. Prevalence and characteristics of Staphylococcus aureus in Connecticut swine and swine farmers. Zoonoses Public Health 60, 234-243.
Parker, D., Prince, A., 2012. Immunopathogenesis of Staphylococcus aureus pulmonary infection. Semin Immunopathol 34, 281-297.
Peacock, S.J., Moore, C.E., Justice, A., Kantzanou, M., Story, L., Mackie, K., O'Neill, G., Day, N.P., 2002. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. Infect Immun 70, 4987-4996.
Peeva, T., Gogov, I., 1983. Presence of enterotoxin D in Staphylococcus aureus strains isolated from food products. Vet Med Nauki 20, 67-71.
PETERSON, A.C., BLACK, J.J., GUNDERSON, M.F., 1962. Staphylococci in competition. II. Effect of total numbers and proportion of staphylococci in mixed cultures on growth in artificial culture medium. Appl Microbiol 10, 23-30.
Porrero, M.C., Wassenaar, T.M., Gómez-Barrero, S., García, M., Bárcena, C., Alvarez, J., Sáez-Llorente, J.L., Fernández-Garayzábal, J.F., Moreno, M.A., Domínguez, L., 2012. Detection of methicillin-resistant Staphylococcus aureus in Iberian pigs. Lett Appl Microbiol 54, 280-285.
Raszyk, J., 1986. [Physical, chemical and biological study of dust from large-scale pig farms]. Vet Med (Praha) 31, 233-244.
Robinson, D.A., Enright, M.C., 2004. Multilocus sequence typing and the evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 10, 92-97.
Schouls, L.M., Spalburg, E.C., van Luit, M., Huijsdens, X.W., Pluister, G.N., van Santen-Verheuvel, M.G., van der Heide, H.G., Grundmann, H., Heck, M.E., de Neeling, A.J., 2009. Multiple-locus variable number tandem repeat analysis of Staphylococcus aureus: comparison with pulsed-field gel electrophoresis and spa-typing. PLoS One 4, e5082.
Schulz, J., Bao, E., Clauss, M., Hartung, J., 2013. The potential of a new air cleaner to reduce airborne microorganisms in pig house air: preliminary results. Berl Munch Tierarztl Wochenschr 126, 143-148.
Schulz, J., Friese, A., Klees, S., Tenhagen, B.A., Fetsch, A., Rösler, U., Hartung, J., 2012. Longitudinal study of the contamination of air and of soil surfaces in the vicinity of pig barns by livestock-associated methicillin-resistant Staphylococcus aureus. Appl Environ Microbiol 78, 5666-5671.
Sergio, D.M., Koh, T.H., Hsu, L.Y., Ogden, B.E., Goh, A.L., Chow, P.K., 2007. Investigation of meticillin-resistant Staphylococcus aureus in pigs used for research. J Med Microbiol 56, 1107-1109.
Shopsin, B., Gomez, M., Montgomery, S.O., Smith, D.H., Waddington, M., Dodge, D.E., Bost, D.A., Riehman, M., Naidich, S., Kreiswirth, B.N., 1999. Evaluation of

protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 37, 3556-3563.
Skalka, B., 1991. [Occurrence of staphylococcal species in clinically healthy domestic animals]. Vet Med (Praha) 36, 9-19.
Smith, T.C., Gebreyes, W.A., Abley, M.J., Harper, A.L., Forshey, B.M., Male, M.J., Martin, H.W., Molla, B.Z., Sreevatsan, S., Thakur, S., Thiruvengadam, M., Davies, P.R., 2013. Methicillin-Resistant Staphylococcus aureus in Pigs and Farm Workers on Conventional and Antibiotic-Free Swine Farms in the USA. PLoS One 8, e63704.
Smith, T.C., Male, M.J., Harper, A.L., Kroeger, J.S., Tinkler, G.P., Moritz, E.D., Capuano, A.W., Herwaldt, L.A., Diekema, D.J., 2009. Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One 4, e4258.
Smith, T.C., Pearson, N., 2011. The emergence of Staphylococcus aureus ST398. Vector Borne Zoonotic Dis 11, 327-339.
Sollid, J.U., Furberg, A.S., Hanssen, A.M., Johannessen, M., 2013. Staphylococcus aureus: Determinants of human carriage. Infect Genet Evol.
Tenover, F.C., Arbeit, R., Archer, G., Biddle, J., Byrne, S., Goering, R., Hancock, G., Hébert, G.A., Hill, B., Hollis, R., 1994. Comparison of traditional and molecular methods of typing isolates of Staphylococcus aureus. J Clin Microbiol 32, 407-415.
Turlej, A., Hryniewicz, W., Empel, J., 2011. Staphylococcal cassette chromosome mec (Sccmec) classification and typing methods: an overview. Pol J Microbiol 60, 95-103.
Turner, G.V., 1982. A microbiological study of polyarthritis in slaughter pigs. J S Afr Vet Assoc 53, 99-101.
Utsui, Y., Yokota, T., 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant Staphylococcus aureus. Antimicrob Agents Chemother 28, 397-403.
van Cleef, B.A., Broens, E.M., Voss, A., Huijsdens, X.W., Züchner, L., Van Benthem, B.H., Kluytmans, J.A., Mulders, M.N., Van De Giessen, A.W., 2010a. High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands. Epidemiol Infect 138, 756-763.
van Cleef, B.A., Verkade, E.J., Wulf, M.W., Buiting, A.G., Voss, A., Huijsdens, X.W., van Pelt, W., Mulders, M.N., Kluytmans, J.A., 2010b. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. PLoS One 5, e9385.
van der Wolf, P.J., Rothkamp, A., Junker, K., de Neeling, A.J., 2012. Staphylococcus aureus (MSSA) and MRSA (CC398) isolated from post-mortem samples from pigs. Vet Microbiol 158, 136-141.
van Duijkeren, E., Jansen, M.D., Flemming, S.C., de Neeling, H., Wagenaar, J.A., Schoormans, A.H., van Nes, A., Fluit, A.C., 2007. Methicillin-resistant Staphylococcus aureus in pigs with exudative epidermitis. Emerg Infect Dis 13, 1408-1410.

van Loo, I., Huijsdens, X., Tiemersma, E., de Neeling, A., van de Sande-Bruinsma, N., Beaujean, D., Voss, A., Kluytmans, J., 2007. Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans. Emerg Infect Dis 13, 1834-1839.

Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2010. Methicillin-resistant Staphylococcus aureus (MRSA) in food production animals. Epidemiol Infect 138, 606-625.

Verkade, E., van Benthem, B., den Bergh, M.K., van Cleef, B., van Rijen, M., Bosch, T., Kluytmans, J., 2013. Dynamics and Determinants of Staphylococcus aureus Carriage in Livestock Veterinarians: A Prospective Cohort Study. Clin Infect Dis 57, e11-17.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant Staphylococcus aureus in pig farming. Emerg Infect Dis 11, 1965-1966.

Weese, J.S., Zwambag, A., Rosendal, T., Reid-Smith, R., Friendship, R., 2011. Longitudinal investigation of methicillin-resistant Staphylococcus aureus in piglets. Zoonoses Public Health 58, 238-243.

Wertheim, H.F., Melles, D.C., Vos, M.C., van Leeuwen, W., van Belkum, A., Verbrugh, H.A., Nouwen, J.L., 2005. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 5, 751-762.

WILLIAMS, R.E., 1963. Healthy carriage of Staphylococcus aureus: its prevalence and importance. Bacteriol Rev 27, 56-71.

Wulf, M., Voss, A., 2008. MRSA in livestock animals-an epidemic waiting to happen? Clin Microbiol Infect 14, 519-521.

Zecconi, A., Scali, F., 2013. Staphylococcus aureus virulence factors in evasion from innate immune defenses in human and animal diseases. Immunol Lett 150, 12-22.

Zhang, C., Song, L., Chen, H., Liu, Y., Qin, Y., Ning, Y., 2012. Antimicrobial susceptibility and molecular subtypes of Staphylococcus aureus isolated from pig tonsils and cow's milk in China. Can J Vet Res 76, 268-274.