Prevention and Control of Methicillin-resistant \textit{Staphylococcus aureus} in Equine Hospitals in Sweden

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Doctoral Thesis
\textit{Swedish University of Agricultural Sciences}
\textit{Skara 2013}
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Abstract

Methicillin-resistant *S. aureus* (MRSA) was first described in 1961 and has since caused nosocomial infections and therapeutic limitations. In Sweden, the first finding of horses infected with MRSA was in 2008. Nosocomial spread of MRSA among horses is a hazard for the patients and those in contact with the animals. Therefore, there is a need to introduce or improve routines for prevention and control of MRSA in veterinary care. MRSA spa-type t011, CC398 caused an outbreak of surgical site infections in horses. MRSA CC398 is associated with livestock and has been reported in horses in Europe. The superficial infections healed without antimicrobial treatment. Longitudinal sampling of post-infected horses showed that all tested negative by time, median 143 days. The most sensitive site to test for MRSA carriage was the nostrils, with a relative sensitivity of 0.91. Due to few sampled cases (n=9) MRSA carriage in horses needs more study. Transmission of MRSA by horses and humans to the environment was shown through environmental screening. In total, 10 of 92 samples were positive. The screening was a useful tool in the implementation of basic hygiene. Rapid response combined with multidisciplinary collaboration was key in the outbreak control. This led to an improved infection control (IC) operation. Infection control procedures used in human health care were mainly applicable, but existing differences between equine and human settings require adapted solutions. Baseline data on IC procedures in three equine hospitals was collected. Overall excellent compliance with dress regulations and personal appearance (no rings or wrist watches, short nails etc.) was shown. Compliance with hand hygiene procedures was poorer. Purchase data per patient of hygiene products were useful to indirectly monitor compliance trends over time. Barriers to compliance were such as inaccessible hygiene products, insufficient knowledge of procedures and high work load. The knowledge presented in this thesis on epidemiology and prevention and control of MRSA in equine medicine can be used in the development and implementation of IC procedures in Swedish equine hospitals. Supplementary multidisciplinary studies of MRSA carriage in horses, species-specific factors affecting IC and, implementation and compliance with IC can develop the topic further.

*Keywords*: horses, equine, methicillin-resistant *Staphylococcus aureus*, MRSA, infection prevention and control

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To my dad, always with me...

Se upp i farleden! här kommer en som har tappat rodret
Allan Edwall
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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:


IV Bergström K. & Grönlund Andersson U. Pre- and post-intervention study of infection control operations in equine hospitals in Sweden (manuscript).

Papers I-III are reproduced with the kindly permission of the publishers.
The contribution of KB to the papers included in this thesis was as follows:

I  Idea, research planning, data collection, data analysis and manuscript preparation.

II Idea, hypothesis, study design, research planning, data collection, data analysis and manuscript preparation.

III Idea, research planning, data collection, data analysis and manuscript preparation.

IV Idea, study design, research planning, data collection, data analysis and manuscript preparation.
## Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BURST</td>
<td>Based Upon Related Sequence Types</td>
</tr>
<tr>
<td>CA-MRSA</td>
<td>Community acquired MRSA</td>
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<tr>
<td>CC</td>
<td>Clonal complex</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
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<tr>
<td>CMRSA</td>
<td>Canadian methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>EMRSA</td>
<td>Epidemic methicillin-resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>HAI</td>
<td>Hospital-associated infection</td>
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<td>HA-MRSA</td>
<td>Hospital associated MRSA</td>
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<tr>
<td>IC</td>
<td>Infection prevention and control</td>
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<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>LA-MRSA</td>
<td>Livestock associated MRSA</td>
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<tr>
<td>MALDI TOF</td>
<td>Matrix-assisted laser desorption/ionization time-of-flight</td>
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<td>MH</td>
<td>Mueller Hinton broth</td>
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<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<td>MLST</td>
<td>Multi locus sequence typing</td>
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<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
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<td>MSSA</td>
<td>Methicillin-susceptible <em>Staphylococcus aureus</em></td>
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<tr>
<td>NaCl</td>
<td>Sodium chloride or salt</td>
</tr>
<tr>
<td>OIE</td>
<td>World Organisation for Animal Health</td>
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<tr>
<td>PBP</td>
<td>Penicillin-binding protein</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PFGE</td>
<td>Pulsed field gel electrophoresis</td>
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<tr>
<td>PMB</td>
<td>Phenol mannitol broth</td>
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<tr>
<td>PVL</td>
<td>Panton-Valentine leukocidins</td>
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<tr>
<td>SCC</td>
<td>Staphylococcal cassette chromosome</td>
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<tr>
<td>ST</td>
<td>Sequence type</td>
</tr>
<tr>
<td>subsp.</td>
<td>subspecies</td>
</tr>
<tr>
<td>TSB</td>
<td>Tryptone soy broth</td>
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1 Introduction

1.1 Historical review of infection prevention and control

Three billion years ago bacteria emerged. Approximately 200,000 years ago, the ‘modern’ human, Homo sapiens, originated in Africa. In this timeline, infection prevention and infection control (IC) has merely started. In the past, rinderpest (cattle plague) killed hundreds of millions of cattle in Europe, Asia and Africa and caused secondary socio-economic effects and death of humans by famine (Wilkinson, 1984). In Italy, Bernardo Ramazzini (1633-1714) expressed ideas about isolation of animals, cleanliness and fumigation of animal houses to cure rinderpest, while Giovanni Maria Lancisi (1654-1720) had an idea that symptoms and dissemination were correlated. When Lancisi grouped animals according to health status and culled those with symptoms, the outbreak abated and the area was free of rinderpest for many years (Blumberg, 1989; Wilkinson, 1984). Approximately 200 years later (1920), a Belgian outbreak of rinderpest was the impetus for international mutual aid in controlling animal diseases, leading to the World Organisation for Animal Health (OIE) in 1924. Rinderpest was declared eradicated worldwide by OIE in May 2011 and by the Food and Agriculture Organization of the United Nations in June 2011. Vaccination was the next important preventive breakthrough. Inoculation with cowpox pus was probably practiced in Africa and Asia prior to the introduction to Europe (Gross & Sepkowitz, 1998), but Edward Jenner (1749-1823) is acknowledged for vaccination as he took the idea to the public (Riedel, 2005). Jenner predicted that general vaccination for smallpox would wipe out the disease and in 1979 smallpox was declared eradicated by the World Health Organization (Miller et al., 2006).

About 50 years later, Ignaz Semmelweis (1818-1865) showed that improved hand hygiene reduced maternal death from puerperal sepsis and is today acknowledged as a progenitor of hand hygienisation. In his time, medical students performed autopsies and entered labour wards without washing their
hands. Later statistical calculations based on data reported by Semmelweis showed significant evidence that: i) Maternal mortality in puerperal sepsis was lower in a Dublin hospital where hand washing was customary than in Semmelweis’ hospital; and ii) after introducing chlorine hand washing, maternal mortality was reduced to about the Dublin level (Noakes et al., 2008). Although preventive measures as described above gradually came into use, the cause of infectious diseases was still unknown. The explanation emerged during the mid and late 19th century, when scientists such as Louis Pasteur (1822-1865) and Robert Koch (1843-1910) demonstrated that microorganisms cause disease (Pasteur et al., 2002; Gradmann, 2001; Koch, 1882). In the beginning of the 20th century, antimicrobial drugs were discovered. Alexander Fleming (1881-1955) is probably best known for his discovery of penicillin (Ligon, 2004). The drug was miraculous and many wounded in World War II survived due to penicillin. Few, if any, listened to warnings of microbial resistance that Fleming mentioned already in his Nobel lecture on 11 December, 1945 (Fleming, 1945). Many antimicrobial substances have been introduced since penicillin, but sooner or later bacteria resistant to these drugs have emerged (Hogberg et al., 2010). The strong focus on elimination of disease-causing microbes and the weaker focus on the transmission factors leading to infection in other hosts have made us over-confident with antimicrobial treatment, resulting in antimicrobial resistance (Humphreys et al., 2009). The growing burden of antimicrobial resistance and that very few new antibacterial classes have been developed have become a worldwide threat to both humans and animals (Cars et al., 2008). We are heading towards pre-antibiotic days. The ‘third epidemiological transition’ is a universal change in infectious disease epidemiology, where antimicrobial resistant bacteria are a strongly influencing global factor (Harper & Armelagos, 2010). In this epidemiological transition, we have to recapture and further develop infection control.

Infection prevention and control (IC) in veterinary medicine includes a wide range of activities applied on herd level, in trade of animals, in wild life and food-related industries and in animal hospital environments. These are intended to control both animal and zoonotic diseases. However, this thesis confines itself to examining the prevention and control of MRSA within equine hospital environments, although comparisons to human and other animal species naturally occur.
1.2 Methicillin-resistant \textit{Staphylococcus aureus} (MRSA)

\textit{Staphylococcus} (\textit{S.}) \textit{aureus} is a commensal bacterial pathogen in humans and several animal species (Fluit, 2012; Feng \textit{et al.}, 2008; Lowy, 1998). Methicillin-resistant \textit{S. aureus} or MRSA emerged during the early 60s (Jevons, 1961), and has since caused concerns due to nosocomial infections in human health care (Kock \textit{et al.}, 2010; Grundmann \textit{et al.}, 2006; Hsueh \textit{et al.}, 2004). However, a significant decrease in human MRSA rates has been reported in seven European countries during recent years, which could be a reflection of successful preventive measures (Heuer \textit{et al.}, 2010 (revised 2011)). Nevertheless, increasing reports of MRSA infections in horses during the past decade have reminded us of the need for IC in equine hospitals too (Schwaber \textit{et al.}, 2013; van Duijkeren \textit{et al.}, 2010; Anderson \textit{et al.}, 2009; Cuny \textit{et al.}, 2008; Leonard & Markey, 2008; Shimizu & Kato, 1979).

1.2.1 Classification of \textit{Staphylococcus aureus}

The prokaryotic domain bacteria are ordered into 30 phyla (lines of development) and further into classes, orders, families and finally genera. \textit{Staphylococcus aureus} belongs to the phylum \textit{Firmicutes}, class \textit{Bacilli}, order \textit{Bacillales} with the family name \textit{Staphylococcaceae} and genus \textit{Staphylococcus}. \textit{Staphylococcus aureus} is a species within the genus. Two subspecies have been described, \textit{S. aureus} subsp. \textit{aureus} and \textit{S. aureus} subsp. \textit{anaerobius}. This thesis deals solely with subsp. \textit{aureus} and from here on, ‘\textit{S. aureus}’ refers to \textit{S. aureus} subsp. \textit{aureus} (De Vos \textit{et al.}, 2009).

1.2.2 Methicillin resistance

Apart from the methicillin resistance, MRSA is similar to methicillin-susceptible \textit{S. aureus} (MSSA). Methicillin belongs to a comprehensive class of antimicrobials, the beta-lactam antimicrobials or beta-lactams. The mode of action is inhibition of bacterial cell wall biosynthesis. The group includes penicillin derivates, cephalosporins, monobactams and carbapenems.

Penicillin-resistant \textit{S. aureus}, caused by acquisition of a plasmid encoding the penicillin-degrading enzyme penicillinase, was first reported in 1942 (Novick, 1963; Munch-Petersen & Boundy, 1962). Methicillin was introduced in 1959 and its mode of action initially made it effective against penicillinase-producing \textit{S. aureus}. However, only two years later a report of methicillin-resistant \textit{S. aureus} came from the UK (Jevons, 1961). The designation MRSA is still in use today, although methicillin has been replaced by other penicillinase-stable beta-lactams for clinical use.

The effect of beta-lactams on bacteria occurs by adhesion to penicillin-binding protein (PBP) on the cell surface of the bacteria. PBP is vital for
bacterial cell wall synthesis and when beta-lactams bind to PBP, cell growth is restrained (Lyon & Skurray, 1987; Georgopapadakou et al., 1982). MRSA carries a gene encoding a different PBP with low affinity to beta-lactams (Georgopapadakou et al., 1982). The adherence of beta-lactam to the cell wall of MRSA therefore fails, the biosynthesis of the peptidoglycan layer in the cell membrane continues and the bacterial cell survives (Berger-Bachi & Rohrer, 2002). Two mec genes, mecA and mecC have been described (Garcia-Alvarez et al., 2011). The latter was identified a few years ago and is not detected by conventional MRSA confirmatory methods (see section 2.3).

Genes encoding antimicrobial resistance are commonly carried on mobile genetic elements, either in the chromosome or a plasmid, which can be transferred both within and between species. In MRSA the mec genes have been identified in the staphylococcal cassette chromosome, SCCmec. There are four general components in SCCmec elements: i) the mec gene complex; ii) the ccr gene complex; iii) a typical nucleotide sequence at both ends of the element; and iv) an integration site for SCC with incomplete inverted repeats located at the 3’-end of orfX (Anonymous, 2009).

1.2.3 Sampling and culture

In clinical bacteriology, culture procedures normally include non-selective medium to allow different pathogens to grow. If there is a direct suspicion of MRSA infection, for confirmation or screening selective medium should be used.

All tests have a risk of false negative or false positive results. Carrying out laboratory procedures according to standard operating protocols is important for all tests. However the performance of a test will never be better than the quality of the specimen, so high quality sampling is an important first step.

Sampling

In the case of infection it is obvious that the infected site should be sampled, but for screening of carriage the sampling site is not as obvious. Nostril sampling has been applied in screening of MRSA in horses (Axon et al., 2011; Tokateloff et al., 2009; Van den Eede et al., 2009; Bagcigil et al., 2007; Vengust et al., 2006; Weese & Rousseau, 2005), most likely based on studies of S. aureus in horses and humans. In earlier studies in horses, coagulase-positive staphylococci were isolated from the nostrils, but also other locations (Kawano et al., 1981; Shimizu & Kato, 1979). When staphylococci isolated from lesions and normal skin of horses were studied, S. aureus (and S. intermedius and S. hyicus) were mostly detected in samples from lesions (Devriese et al., 1985). In a study comparing sampling of nostrils and eight
skin sampling sites for MRSA detection the most sensitive site was the nostrils (Van den Eede et al., 2012). Furthermore, it was recently reported that the nasal vestibulum was the best sampling site of three within the nostril cavity of the equine (Van den Eede et al., 2013). In humans, the prime ecological niche for *S. aureus* is the anterior nares (Kluytmans et al., 1997; Williams, 1963). However, in MRSA screening, sampling of several locations beside the nares, such as the perineum and throat, are recommended to increase sensitivity (Senn et al., 2012; Andersen et al., 2010; Bitterman et al., 2010).

Despite differences in culture methods between the studies cited above, a rational conclusion is that *S. aureus* and MRSA are most often isolated from the nostrils and/or skin lesions in horses. Although it was not until recently that a study actually compared different sampling sites.

**Solid culture medium**

The species *S. aureus* grows well on non-selective medium, such as 5% blood agar. A basic feature which selects for *S. aureus* is salt, as in mannitol salt agar (Brown et al., 2005). Mannitol also supports *S. aureus*, but is not specific.

Adding certain antimicrobials to the medium will select for MRSA. Varied concentrations of NaCl and antimicrobials in the medium may play a role for the sensitivity level. The requirements can also differ between strains (Brown et al., 2005). Strains of EMRSA-16 (corresponding to ST22) are less tolerant to high salt concentrations (Jones et al., 1997).

Chromogenic agar is a commercial medium for detection of different bacteria species (and yeasts) where colonies grow with a specific colour. The medium has been adapted for *S. aureus* and specific MRSA detection. Chromogenic, cefoxitin-based medium is selective, specific and offers a short turn-around time on direct inoculation (Struelens et al., 2009). Cefoxitin-containing agar has also been reported to perform well in detection of MRSA in other studies (Perry et al., 2004; Skov et al., 2003; Felten et al., 2002).

**Enrichment broth**

In general, sensitivity increases when selective enrichment broth is used prior to plating. The principles for enrichment of MRSA are as for the solid medium (Brown et al., 2005). Example of broths used is Tryptone soy broth (TSB) with NaCl, mannitol and antimicrobials, and phenol red mannitol broth with antimicrobials (van Duijkeren et al., 2010; Graveland et al., 2009; Vos et al., 2009). Another example is Brain Heart Infusion broth containing colistin and nalidixic acid, which was used in screening for MRSA in horses (Van den Eede et al., 2009). Other antimicrobials utilised are azetreonam, cefizoxime, oxacillin and cefoxitin. Minor adjustments in the formula of the broth between
studies are frequent, as concentration of antimicrobial substances and NaCl (van Duijkeren et al., 2010; Vos et al., 2009; Bocher et al., 2008).

Mueller Hinton (MH) broth with NaCl but without antimicrobials as a pre-enrichment step prior to the selective broth has been used to further increase sensitivity in specimens (Agerso et al., 2012; van Duijkeren et al., 2010; Anonymous, 2007).

Which culture method to use?
An optimal method to culture MRSA cannot be decided on the basis of the existing evidence. Comparisons of culture methods in equine settings have shown that adding an enrichment step results in more MRSA-positive samples than direct culture (van Duijkeren et al., 2010; Van den Eede et al., 2009). Comparison of culture methods for samples from other animal species and humans also shows increased sensitivity by enrichment (Graveland et al., 2009; Bocher et al., 2008).

The overarching advantage of culture is that the whole bacterial cell is harvested. Culture generally has high specificity, while sensitivity is lower. The sensitivity is also affected by the sample quality, the quantity of MRSA, co-occurrence of other bacteria etc. Culture is slow and with enrichment even more so, not ideal in clinical cases or admission screening.

1.2.4 Identification of the species S. aureus

Phenotyping
Macroscopic appearance of colonies, Gram staining of bacterial cells, microscopic appearance and biochemical tests are typical phenotyping tests for bacteria. S. aureus is a Gram-positive, coagulase-test positive coccus (De Vos et al., 2009). Phenotyping tests are strong indicators for the species, but false negative and positive results can occur.

PCR
Polymerase Chain Reaction (PCR) amplification of the specific S. aureus nuc gene is considered a highly reliable method when performed on grown colonies (Brakstad et al., 1992). PCR methods are in general fast and can also detect dead bacteria, which could be both an advantage and a disadvantage.

MALDI-TOF
Matrix-Assisted Laser Desorption/Ionization Time-of-Flight, or MALDI-TOF, is a method based on mass spectrometry. It is fast and relatively cheap, and generally gives better reproducibility than phenotyping tests, but single
colonies are still needed and culture is required (Fenselau & Demirev, 2001). The method has future potential to give information on subspecies and even individual organisms. The advantage of the method so far is the automated process and that time to typing goes from days to minutes.

1.2.5 Detection of methicillin resistance

**Antimicrobial susceptibility testing**

In clinical bacteriology, high MIC values for cefoxitin and/or oxacillin in *S. aureus* indicate methicillin resistance. The recommended cut-off for detection of methicillin resistance is an MIC value > 2 mg/L for oxacillin and/or > 4 mg/L for cefoxitin, according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2013). Still, there is no clearcut boundary in MIC values between MRSA and MSSA, with the risk of false negative and positive results. Therefore further analyses are needed, such as confirmation of the *mec* gene or the specific PBP protein.

**Latex agglutination**

Latex agglutination is a slide agglutination assay. To detect MRSA specific monoclonal antibody directed towards the PBP2a antigen is used (van Leeuwen *et al.*, 1999). False positive results occur, e.g. coagulase-negative staphylococci encoding the protein. False negatives could be MRSA containing the gene but with the protein not expressed. Moreover, this test does not cover the protein encoded by *mecC*. The method is suitable for use when access to laboratory equipment is limited (van Leeuwen *et al.*, 1999).

**PCR**

PCR amplification by the *mec* genes combined with the *nuc* gene is a highly reliable method when performed on grown colonies (Kearns *et al.*, 1999). PCR detection of MRSA directly from specimens is also possible, but the PCR in question does not cover *mecC* (Huletsky *et al.*, 2004). The turn-around time is hours, instead of days for culture.

1.2.6 Genotyping and nomenclature

Dependent on if long-term and global or short-term and local comparison is of interest different typing methods can be used. A method investigating genetic variations that accumulates slowly suits the long-term, global perspective. Consequently method finding variations that accumulates rapidly is of interest for such as outbreak investigations.
Multi Locus Sequence Typing

Multi Locus Sequence Typing (MLST) is a standardised sequencing of seven housekeeping genes present in all *S. aureus*. Each genes’ unique sequence put together gives the allelic profile or the sequence type (ST) (Enright *et al*., 2000). The ST type is given a number dependent on the allelic profile which can be found in the MLST data base (MLST, 2013). The method is used for long-term/global comparison, as the sequenced genes change by natural mutation, i.e. variations accumulate slowly. The Based Upon Related Sequence Type (BURST) algorithm can be used to identify related genotypes, referred as clonal complex (CC) (Feil *et al*., 2004). The method is laborious, costly and has limited discriminatory power therefore *spa*-typing is an option.

Spa-typing

*Spa*-typing is a standardised sequencing of short polymorphic variable-number tandem repeats in a specific region of the *S. aureus* protein A (*spa*) gene. The method is discriminatory enough for outbreak investigations and variants occur frequently in the *spa* gene (Harmsen *et al*., 2003). In the Ridom SpaSever information of different *spa*-types can be found and international comparison is possible (RidomSpaServer, 2013; Harmsen *et al*., 2003). The designation is *spa*-type and a combination of ‘t’ and a figure, e.g. t011 or t064.

Pulsed Field Gel Electrophoresis

Pulsed Field Gel Electrophoresis (PFGE) involves digestion of bacterial DNA by a restriction enzyme. The DNA fragments are allowed to wander in a pulsed electric field gel. A strain specific band pattern is shown as fragments move according to lengths. The advantage with PFGE is its highly discriminatory power as minor genetic variations shows. A disadvantage with PFGE is poor reproducibility. A harmonised consensus PFGE protocol for typing of MRSA has partly resolved this (Murchan *et al*., 2003). It is laborious and slow, and some lineages are not typeable by the ‘gold standard’ enzyme, *Sma*I.

SCCmec

*SCCmec* typing is sequencing of variable regions of the *mec* gene by PCR amplification. *SCCmec* classes are based on variation in the *mec* and *ccr* complex and are named in chronological order after discovery (Anonymous, 2009 ). Subtypes of the *SCCmec* have been designated due to variations in junkyard- or J-regions within the cassette. The method gives information of evolutionary origin and spread of MRSA. It is a rapid, discriminatory method with harmonised nomenclature.
PVL

Panton-Valentine Leukocidins (PVL) is mentioned as considered a clinical virulence marker. The toxin causes severe tissue necrosis in otherwise healthy people (Zetola et al., 2005; Miles et al., 2002), although its role in virulence has been questioned (Dumitrescu et al., 2011; Otto, 2010). Detection is by PCR amplification of two genes encoding PVL.

Nomenclature

A simplistic epidemiological grouping of MRSA is hospital-associated (HA) MRSA, community-acquired (CA) MRSA and livestock-associated (LA) MRSA. CA-MRSA has also been subdivided into health care-associated CA-MRSA and ‘true’ CA-MRSA. The different groups have no definite correlation to molecular subdivision of lineages.

The genotyping nomenclature of MRSA has not been finally standardised. There is nomenclature for S. aureus built on the MLST, designated ST lineages and the clustering into clonal complex (Feil et al., 2004). Epidemic clones by PFGE analysis is another. In the United States such have been named USA 100, 200, etc., in Canada CMRSA 1, 2, 3 etc. and in the United Kingdom (UK) EMRSA 1 to 14 (Stefani et al., 2012).

1.3 MRSA in horses

Increasing reports of MRSA in horses have been published in the past decade (Cuny et al., 2010; van Duijkeren et al., 2010; Anderson et al., 2009; Van den Eede et al., 2009; Leonard & Markey, 2008; Weese et al., 2006; Weese et al., 2005b; Shimizu et al., 1997). Epidemiological knowledge of MRSA in horses is required due to the risk of infection and dissemination between horses, other animals and humans.

1.3.1 Occurrence

The first report of MRSA in horses was in Japan, in a stallion with a skin lesion and 13 brood mares with metritis (Shimizu et al., 1997). All isolates except one were shown by PFGE to be of the same origin, indicating cross-infection among the breeding animals. MRSA prevalence rates of between zero and 11% have been reported on horse farms and/or at admittance to equine hospitals in Australia, Canada and various European countries (Axon et al., 2011; Tokateloff et al., 2009; Van den Eede et al., 2009; Burton et al., 2008; Vengust et al., 2006).
**MRSA CC8**

MRSA CC8 has been detected in both Europe and North America, and has become a frequent type in horses. In the UK/Ireland, Belgium, Austria, the Netherlands and Germany, the spa-types t008, t020, t036, t064 and t451, all connected to CC8, have been reported (van Duijkeren et al., 2010; Walther et al., 2009; Cuny et al., 2008; Moodley et al., 2006). In North America, MRSA CC8, spa-type t064 classified by PFGE as Canadian MRSA-5 or USA500 is common (Anderson et al., 2009; Tokateloff et al., 2009; Weese et al., 2006). The Canadian clone has been proposed to be adapted to horses (Weese & van Duijkeren, 2010). The European findings referred above seem in agreement with the idea of horse adapted strains.

**MRSA CC398**

In Europe, another commonly detected MRSA in horses is CC398, also named livestock associated or LA-MRSA (Cuny et al., 2008). CC398 is a significant clone in livestock and was first reported in pigs in Europe, but also occurs in other species such as cattle, poultry and humans (Paterson et al., 2012; Lewis et al., 2008; Nemati et al., 2008; Monecke et al., 2007). LA-MRSA has been reported from other parts of the world too, in species such as pigs and humans (Osadebe et al., 2013; Stegger et al., 2010). Recent phylogenetic analyses of CC398 MSSA and MRSA strains strongly suggest that a human MSSA strain was the ancestor of MRSA CC398 (Price et al., 2012). The human MSSA lost phage-carried virulence genes and gained tetracycline and methicillin resistance.

A substantial proportion of the MRSA CC398 found in horses in Europe is of spa-type t011 (Van den Eede et al., 2013; van Duijkeren et al., 2010; Van den Eede et al., 2009; Cuny et al., 2008).

The dominant types, CC8 and CC398, are also those detected in the Swedish equine population. Mainly CC398 (spa-type t011) while CC8 (spa-type t064) has been sporadically detected (SVARM, 2012).

**Less common MRSA types**

Less frequently reported MRSA types in horses are USA100/CMRSA-2, corresponding to CC5 (Weese et al., 2006). In an Israeli equine hospital outbreak, ST5, spa-type t535 was detected (Schwaber et al., 2013). One horse isolate of CC15, spa-type t084, was also reported (Stegger et al., 2010).

Mec-typing is far from always performed in horse studies, but in CC398 isolates from Austria and Germany, SCCmec IVa, IVd and V have been reported (Cuny et al., 2008; Witte et al., 2007).
1.3.2 Clinical aspects

Infections with MRSA in horses appear to be primarily opportunistic (van Duijkeren et al., 2010; Anderson et al., 2009; Cuny et al., 2008; Weese et al., 2006). In horses, *S. aureus* including MRSA causes different types of dermatitis, skin and wound infections but also abscesses, arthritis, metritis, eye infections and various invasive infections (Schwaber et al., 2013; van Duijkeren et al., 2010; Cuny et al., 2008; Weese et al., 2006; Shimizu et al., 1997; Devriese et al., 1985; Raus & Love, 1983). According to these studies surgical site infections dominate, with spa-type t011 being a commonly reported cause of infections in equine clinics and hospitals.

MRSA isolates found in horses in Sweden have mostly been susceptible only to erythromycin, clindamycin and fusidic acid (SVARM, 2012). Resistance to three or more classes of antimicrobial agents by phenotypic susceptibility testing means that an animal isolate is defined as multi-resistant (Schwarz et al., 2010). None of these antimicrobials is authorised for use in horses in Sweden and infections will be difficult to treat if antimicrobials are needed. For example erythromycin is also known to cause fatal diarrhoea in horses (Gustafsson et al., 1997).

1.3.3 Risk factors

Individual horses testing positive for MRSA on admission to hospital are more likely to suffer from clinical MRSA infection than non-carriers (Weese et al., 2006). Subtypes of MRSA found in horse nostrils on admittance to hospital was found in later infections (van Duijkeren et al., 2010). Administration of ceftiofur or aminoglycosides is a risk factor associated with becoming MRSA-positive during hospitalisation (Weese et al., 2006). Factors reported to be significant for a horse testing MRSA-positive on admittance to hospital are: history of MRSA carriage, coming from a test-positive farm, admission to a foal watch programme or to a service other than surgery, and antimicrobial exposure to penicillin or trimethoprim-sulfa within 30 days of admission (Weese & Lefebvre, 2007). A significant association between infected incision sites and nosocomial MRSA has also been reported (Anderson et al., 2009).

The risk factors found in human medicine are similar. For example, history of MRSA carriage or infection, frequent hospital admissions or recent admission to a hospital with a known high prevalence of MRSA is some of the risk factors reported in humans (Coia et al., 2006). Other examples include an association with MRSA carriage due to fluoroquinolone exposure (Harbarth et al., 2000) and a reported causal relationship between antimicrobial use and MRSA acquisition (Muller et al., 2003).
1.3.4 Zoonotic aspects

An increased risk of staff at equine hospitals becoming contaminated or colonised by MRSA and an associated increased risk of becoming infected or spreading the MRSA further have to be considered (Schwaber et al., 2013; van Duijkeren et al., 2010; Cuny et al., 2008; Hanselman et al., 2006). In a Dutch equine hospital, the same spa-type, t011 and the related t2123, was present in infected horses and staff (van Duijkeren et al., 2010). In a recently published study of MRSA in an Israeli equine hospital, 12 of 84 horses (14.3%) and 16 of 139 personnel (11.5%) were MRSA carriers of spa-type t535, ST 5 (Schwaber et al., 2013). In addition, the risk of MRSA carriage was greater in equine veterinarians and full-time technicians than in part-time technicians and personnel not working with horses.

Human infections of MRSA CC398 occur (Kock et al., 2013). However, in general human infections are considered uncommon (Graveland et al., 2011a; van Cleef et al., 2011).

As MRSA is a zoonosis, it concerns both human and veterinary medicine. Swedish human and veterinary health authorities collaborate in zoonotic epidemiological matters. According to the Communicable Diseases Act (SFS 2004:168), MRSA is notifiable in humans and contact tracing is mandatory. The Swedish Work Environment Authority (SWEA) makes assessments of microbiological hazards by inspections at workplaces and requires any risks to staff to be dealt with.

1.4 Infection prevention and control

Infection prevention and control is a huge topic in the veterinary aspects mentioned in Chapter 1. However, in this thesis it is viewed from the hospital perspective, in relation to the occurrence of nosocomial MRSA infections in horses. IC procedures in human hospitals are applied to reduce nosocomial infections and dissemination of pathogens, for the safety of patients and staff. IC operations in animal hospitals are less well studied, so some basic scientific findings and experiences from human medicine are therefore considered.

1.4.1 Nosocomial infections

The term nosocomial infection means infection acquired in a hospital, another name being hospital-associated infection (HAI). The exact definition is comprehensive, as it includes criteria for e.g. diagnosing different infections. According to the National Healthcare Safety Network, CDC, USA, a summary of the definition of HAI could be an infection not present on admission and revealed on the third calendar day of admission to the facility (admission is day
1) (CDC, 2013). The upper time limit given varies, for example for deep incisional surgical site infection (SSI) it is 30 or 90 days depending on operation, while for superficial incisional SSI it is 30 days.

The National Board of Health and Welfare (Socialstyrelsen) in Sweden has defined HAI, freely translated as: “Each infectious condition that affects patients following hospitalisation, whether the pathogen is derived from health care or the patient itself and whether the infectious condition is disclosed during or after care” (Socialstyrelsen, 2011). The definition includes more, but these are without relevance in the present context. In veterinary medicine there is no consensus definition of nosocomial infection.

In general, nosocomial infections in human health care cause individual suffering and even death, prolonged hospital stay and increased costs for society, hospital and the individual (Lamarsalle et al., 2013; de Gouvea et al., 2012; Lipp et al., 2012). Studies of the frequency and impact on horses of nosocomial infections in equine medicine are currently lacking.

Dissemination of nosocomial infective agents is influenced by in principle the same factors in animal and human hospitals. Variables such as prevalence, dose of infection and virulence of the agent, flow and number of patients, early or late diagnosis of infection, design of the setting and isolation capacity, level of basic hygiene applied by staff, etc. all have an effect. As hospitalised individuals are likely to be more often contagious and/or susceptible to infections than individuals outside hospital and as they are gathered in a relatively small area, the infection pressure can be too high for the individual, especially if IC fails. Hospitalised horses and humans are subject to comparable suppressing factors, e.g. the stress of being in an unknown environment (the hospital), stress from transportation to the hospital, underlying disease, a change in diet, invasive procedures and antimicrobial use. To reduce the spread of infectious disease, the transmission route also has to be known, e.g. droplets, aerosol, faecal-oral, etc.

1.4.2

Almost one and a half centuries of intervention studies have generated evidence-based hygiene procedures such as hand hygiene and antiseptic precautions effective in preventing nosocomial infections. For some procedures there is contradictory evidence, but a precautionary approach seems wise. In Sweden, Socialstyrelsen regulates basic hygiene within human health care in its “Regulations on basic hygiene in health care, etc.” (SOFS 2007:19).
**Hand hygiene**

Hand hygiene is deemed the single most important measure for prevention of nosocomial infections (WHO, 2009; Boyce & Pittet, 2002). According to Socialstyrelsen, hand disinfectants should be applied and if visible dirt is present hands should be washed prior to disinfection (the exact routine is explained in SOFS 2007:19). Products containing alcohol are recommended today, as these have shown to be efficient in preventing transmission of many (but not all) nosocomial infections and are quicker to apply. Those with moisturising formulas is milder to the skin (Sax et al., 2007). The disadvantage with disinfectant agents such as alcohols, chlorhexidine, iodophors, etc. is the lack of effect on spore-forming bacteria, e.g. *Clostridium difficile*. Plain soap and water and use of gloves are recommended in such cases (Boyce & Pittet, 2002). There is no universal formula covering all pathogens.

**Personal appearance**

Hands and forearms should be free of rings and watches (SOFS 2007:19). It has been shown that jewellery, such as finger rings, nose and ear piercings, significantly increases surface bacterial counts *in situ* and especially after removal (Bartlett et al., 2002). A study of dentists showed a greater number of bacteria under rings and wrist watches than on skin on fingers without rings (Field et al., 1996). Hand disinfection is easier to execute without such items and rings may also damage gloves when used.

Nail polish is another issue, e.g. increased numbers of bacteria on the fingernails of nurses after surgical hand scrubs were noticed on chipped fingernail polish or polish worn longer than four days (Wynd et al., 1994). However, a Cochrane review found no evidence that removing nail polish prevented wound infection after surgery (Arrowsmith & Taylor, 2012).

Tying long hair back to avoid contamination of the hair seems so obvious that evidence might be unnecessary.

**Gloving, gowns and other barriers**

Disposable gloves should be used when there is a risk of becoming contaminated with body fluids or other biological material (SOFS 2007:19). A comparison of contamination per minute of patient care when gloves were used compared with bare hands showed an average of 3 colony forming units (CFU) and 16 CFUs contamination per minute, respectively (Pittet et al., 1999). The incidence of *C. difficile* diarrhoea was reduced from 7.7 cases/1,000 patient discharges to 1.5 cases/1,000 discharges by introduction of vinyl gloves as a routine when handling body substances (Johnson et al., 1990).
Gowns should be worn in case of contact with body fluids or other biological material, according to Socialstyrelsen (SOFS 2007:19). Studies on the use of gowns and preventive action on patient-to-patient transmission of nosocomial infections have shown varied results (Rutala & Weber, 2001). Most studies compare different types of gowns.

Isolation of contagious patients must be considered evidence-based by long and successful tradition, including in veterinary medicine, e.g. by Lancisi mentioned in Chapter 1.

Cleaning and surface disinfection
Contamination of surfaces and items with MRSA has been demonstrated (Coughenour et al., 2011; van Duijkeren et al., 2010; Weese et al., 2004) and S. aureus, including MRSA, can persist on dry inanimate surfaces for months (Kramer et al., 2006). In human health care, non-critical surfaces such as floors are treated differently from surfaces in close proximity to the human patient, as bed rails or items divided into semi-critical and critical surfaces from a transmission point of view (Rutala & Weber, 1999). The latter are considered to pose a higher risk of transmission of pathogens to the patients and are consequently subject to more intensive disinfection than floors.

Disinfectants may be an irritant for humans and hazardous to the environment, and specified safety regulations should be complied with.

Surveillance
Quality monitoring of the IC operation expressed in ‘The Prevention of HAI’ from Socialstyrelsen (Socialstyrelsen, 2006), include: (i) Structural quality, such as enough resources for the operation; (ii) process quality, such as written procedures, etc.; and (iii) quality of performance, such as compliance and presence of HAI.

1.4.3 Implementation and compliance
Implementation comprises introduction of methods or procedures in ordinary activities. Accurately executed implementation should ensure that what has been introduced is also used and conducted as intended. The actual implementation process has been studied in less detail than measures of compliance and barriers to compliance. However, it seems of growing interest (Higgins & Hannan, 2013; Sax et al., 2013; WHO, 2009).

Compliance with especially hand hygiene procedures is a problem in human health care (Yawson & Hesse, 2013; Boyce, 2011; Erasmus et al., 2010; Struelens et al., 2006). A review concluded that high activity level was correlated with lower compliance and that physicians had lower compliance
with hand hygiene in general compared with nurses (Erasmus et al., 2010). Those two factors show the complexity of barriers to compliance. One is easier to respond to (stress), while the latter indicates more diffuse causes, such as attitudes or social norms. Consequently, implementation and compliance need in-depth studies. In a recent mixed-method evaluation study of implementation of infection control, best practices in intensive care units throughout Europe in different cultural milieu of varying economic, political and health care level are described (Sax et al., 2013).

Methods for monitoring compliance used in human health care are indirect measurements of purchases, direct observations by trained and validated observers and self-assessment (Boyce, 2011; Gould et al., 2011; Haas & Larson, 2007; Pittet et al., 2006).

1.4.4 Differences between equine and human hospitals

Various procedures adopted by human medicine are likely to be effective in equine hospitals too. However, differences such as the species in question, hospital environment and infective agents have to be considered when procedures are transferred.

Direct transmission

Direct transmission between individuals should be easier to avoid in equine compared with human hospitals, as horses are generally not allowed to move freely in the hospital. Established procedures for accurate patient flow have to be in place and seem a clear cut, cheap and simply achieved preventive procedure at any equine hospital. On the other hand, restricted flow means that the horses remain in their stall, where they and their feed are in close contact with body wastes, so cleaning and disinfection between patients is important.

Hospital environment

Equine medicine has gradually developed specialism similar to those in human health care, but separate units within the physical hospital building are less common. Isolation, intensive care, neonatal and surgery units may be the most common separate units, while others have either mixed areas or special examination rooms but not whole units.

Rough floorings in equine hospitals are a persistent non-solved disadvantage. They are required to prevent horses from slipping, but are difficult to clean and sanitise. As horses also use the floor for lying, it has to be considered a critical surface of the same level as the bed rail in a human hospital (Rutala & Weber, 1999). Also the manure must be taken care of in a safe way. Porous stall walls made of wood are another surface that is difficult
to sanitise. Huge areas to clean and high costs if done by hand have led to the use of high pressure washing, with a risk of spreading microbes through aerosols.

The climate in Sweden means low temperatures during winter in equine hospitals and short sleeves can be challenging to maintain.

**Hair coat**

Body wastes and dust attach to the hair in the horse coat. In the case of surgery, especially emergency surgery with less or no time for cleaning, this is a problem. On planned hospital visits, a requirement might be introduced that the horse should be clean on admission. Staff handling dirty horses also gets dirty hands that are in need of washing with soap and water. However, frequent washing could cause dry and irritated skin, which is unfavourable from a sanitary view (Kampf & Löffler, 2007).

**Personnel**

The risk to staff when handling patients is lower than in human medicine, due to mainly species-specific agents. However, zoonotic infective agents, such as MRSA and salmonella, are a risk. Possible differences in the education levels of staff and a past history of IC might also influence.

1.4.5 Prevention and control of MRSA in equine hospitals

Prevention and control of MRSA in equine hospitals is poorly studied.

**Screening on admission**

Screening on admission would require isolation of the horses until the test result arrives. Since the turn-around time of today’s available screening tests is long, isolation can also be long. The risk of false negative testing has to be considered and requires knowledge of optimum sampling site/sites and performance of test methods. General screening for MRSA has not been customary in Swedish equine hospitals but regardless of screening or not, IC procedures have to be complied with.

**Hand hygiene and other basic hygiene**

MRSA dissemination by humans in equine hospitals has been reported (van Duijkeren *et al.*, 2010; Weese *et al.*, 2004). Hand hygiene is deemed the single most important measure for prevention of nosocomial infections in human health (WHO, 2009). Contaminated hands are pointed out as a key route of MRSA transmission within veterinary hospitals (Leonard & Markey, 2008).
The use of gloves and, if needed, gowns when handling infected wounds is a basic hygiene measure in human as well as veterinary medicine, but has to be accurately applied. Differences in glove materials and transfer of MRSA by use of dry gloves have been demonstrated. Nitrile gloves showed the lowest transfer rates (Moore et al., 2013), but absorption of simulated body fluids altered the bacterial transfer and it was significantly increased for all glove types. Hence, it is important to change gloves between operations.

Sanitation of fomites potentially carrying MRSA is another issue to consider based on the fact that MRSA has been detected on mobile phones, twitches, muzzles and medical equipment in veterinary hospitals (Julian et al., 2012; Weese et al., 2004). After routine cleaning of computer keyboards in a veterinary clinic, oxacillin-resistant *S. aureus* (i.e. possibly MRSA) was still detected, although in reduced amounts (Bender et al., 2012).

Cleaning of horses is another aspect, as MRSA has shown good survival in dust (Oie & Kamiya, 1996). Dust in the horse coat is inevitable and precautionary cleaning of patients could be recommended. It has also been shown that humans with respiratory tract infection or colonisation shed viable MRSA into the air of their room (Gehanno et al., 2009).

Footwear hygiene should not be neglected, as *S. aureus*, including MRSA, persists on dry inanimate surfaces for months (Kramer et al., 2006).

**Isolation**

Nosocomial spread of MRSA between horses during hospitalisation has been shown or been suspected in previous studies (Schwaber et al., 2013; van Duijkeren et al., 2010; Weese et al., 2006). This suggests that known MRSA carriers should be contact-isolated from other patients, as should infected cases. How strict the isolation should be to be effective requires more studies, but a precautionary principle seems wise. In strict isolation units, precautions normally applied include protective overalls/gowns, clothes, caps and boots donned before entry to an isolated horse.

**Other measures**

The flow of horses and horse owners within the hospitals should be reviewed and in case of weak points revised.

Vector control should not be overlooked, as in farms with pigs and veal calves MRSA ST398 or LA-MRSA was isolated from rats (van de Giessen et al., 2009) and rodents, birds and other pests can be found in equine hospitals.

In Sweden, all cases of MRSA detected in animals are notifiable since 1 January 2008 (SJVFS 2007:90).
2 Aims

The overall aim of this thesis was to gather knowledge required to support prevention and control of MRSA in horses in Swedish equine hospitals. Specific objectives were to:

- Confirm and describe the first nosocomial outbreak of MRSA in horses.
- Identify a reliable sampling site for detection of MRSA carriage in horses.
- Introduce environmental screening as a tool in prevention and control of MRSA.
- Analyse preventive and control interventions introduced to curb an MRSA outbreak.
- Study the effect of intervention on compliance with infection and control procedures in equine hospitals.
3 Materials and methods – considerations

3.1 Summary of study design

- Paper I is a retrospective descriptive outbreak study with genetic analysis of MRSA isolates.

- Paper II is an observational study with recurrent sampling of horses after MRSA infection.

- Paper III is an ambidirectional descriptive study of courses and interventions to curb an outbreak.

- Paper IV is an ambidirectional descriptive pre- and post-interventional study of infection control.

3.2 Study Material

The MRSA studied in Paper I comprised all isolates detected in horses in Sweden in the study period of almost two years, June 2008 to February 2010. The isolates originated from wound infections in an outbreak (n=6), from other clinical sampling (n=4) and from nasal screening (n=2). Inclusion of the MRSA horses after the outbreak was for epidemiological investigation. Medical records, the hospital surgery notes and outbreak investigation notes were used to obtain information on the infected cases.

The longitudinally sampled horses in Paper II were all the available cases (n=9) that had suffered from MRSA infection. Two aged 4 and 6 months and seven >3 years at the start of the study. The study period was between October 2008 and October 2011. MRSA-positive controls without preceding MRSA infection could not be collected due to the low occurrence. Horses in close
contact with those studied were considered potential indicators for dissemination, but only a small caseload was collected.

In Paper III, an outbreak hospital, the Swedish University Teaching Hospital, was studied. IC policy documents, medical records, notes from meetings and cost estimates from the hospital were collected from June 2008 to April 2010.

The equine hospitals included in Paper IV were those willing to participate. By Swedish standards they had a large case load, approximately 5000 visits and 480 surgery patients a year on average, although figures differed somewhat between years. The voluntary participation implies a selection bias, as it is likely that the managers of such hospitals are interested in the topic and therefore might be ‘better’ than average. Controls were not included because of dissimilarities between equine hospitals. Purchase of hand sanitisers and disposable gloves, data on patient numbers and observation data were collected. The total study period ranged between 2008 and 2011, with different start dates for the participating hospitals.

The veracity of data collected by examination of documents in all studies (Papers I-IV) had to be taken for granted and errors in the documents could have biased the results. When possible, this was avoided by triangulation, e.g. comparing data with other sources, discussions with the person logging the data and evaluation by the co-authors or managers at the hospitals.

3.3 Molecular methods

Comparison of MRSA isolates by spa-typing was performed in Papers I-III, as it is standardised, discriminatory and has harmonised nomenclature (Harmsen et al., 2003). MLST analysis of representative isolates was also performed (Enright et al., 2000). PFGE was performed to compare short-term genetic relationships between the MRSA isolates from horses and environmental isolates (Papers I and III) (Tenover et al., 1995). In principle, the protocol of Murchan et al. (2003) was used. Though, because of methylation at the Smal recognition sites in MRSA CC398 (Bens et al., 2006) we used CfrI and Apal enzymes instead. These enzymes have been successfully employed by others in MRSA CC398 isolates (Argudin et al., 2010; Bosch et al., 2010; Rasschaert et al., 2009).

3.4 Longitudinal sampling

Sampling of horses for carriage in their home stables in Paper II was subject to challenging uncontrollable factors. However, as reality is normality, such
studies are needed to understand epidemiology. Efforts should be devoted to defining possible biases and confounders to be considered when evaluating the results. One possible bias was the risk of transient contamination that could be mistaken as carriage. However, transient contamination was likely to be lower in our study than if studied in a high prevalence environment. This was an advantage weighed against the risk of collecting too few cases. We wanted to test whether and for how long MRSA can be detected post-infection in horses and the hypothesis that nostrils would be most sensitive.

The allowance of different starting points related to the MRSA diagnosis for the cases and the fact that one case which was unavailable for sampling for a long time was not excluded could be questioned (Table 1, Paper III). However, as this was the first longitudinal study of MRSA carriage in horses, it was important to keep as many cases as possible and deal with the shortcomings.

3.4.1 Definitions
Since there was no ‘gold standard’ available, a horse was considered MRSA-positive if any sampling site tested positive. The use of two consecutive negative samplings as the definition for decolonisation was an idea taken from an MRSA study on equine farms (Weese & Rousseau, 2005). To verify if this definition was valid, we continued sampling according to the agreed study plan after the cases had fulfilled the definition.

3.4.2 Sampling sites
Nostrils and previously infected sites were the primary sampling sites. The corner of the mouth was chosen as reference to the nostrils, since it is situated near the nostrils at the border of skin and mucosa. The pastern was any skin site not infected, as reference to the previously infected site. The perineum was chosen as used in human MRSA screening (Senn et al., 2012; Andersen et al., 2010) to test if valid in the equine. The sampling within the nostrils was on the border of skin and mucosa. This was empirically chosen, as *S. aureus* is found on both skin and mucosa, and the anterior nares are the prime ecological niche in humans (Williams, 1963). The throat has been suggested as a site to include in multiple body site sampling for *S. aureus* and MRSA in humans (Bitterman et al., 2010). This was never an option in our study because of the species differences. Collecting a throat sample from a horse is more complicated due to the anatomy and it also needs the horse to be restrained during sampling.
3.5 Environmental screening of MRSA

Not surprisingly, a ‘gold standard’ operating procedure cannot be found for environmental MRSA detection, as external conditions in the environment differ between studies and also in real life.

3.5.1 Requirements for the protocol
The main objective was to evaluate environmental screening as a method to demonstrate dissemination of MRSA and its value in routine checks of the IC operation. It had to allow: testing of large areas and many items, testing after routine cleaning and disinfection, uncomplicated collection of samples and identification of indirect contact transmission routes and items difficult to sanitise, all at a reasonable cost.

The screening protocol decided on in paper III was empirically based on experiences, common knowledge and other studies, and developed to suit the circumstances. Ideally, this protocol should have been evaluated prior to use, but time did not allow this.

3.5.2 Decided protocol
In order to cover our requirements, the following were included in the screening protocol:

Firstly, two categories of sampling area were decided: (i) Where people only had access; and (ii) where horses and people had access.

Secondly, surfaces and items where MRSA would be likely to occur were chosen for sampling. The relatively infrequent sampling was considered adequate for the purposes of the study and if proven suitable for routine checks of IC operation, it would be reasonably practical and cost-effective.

Thirdly, the sampling device of pre-packed swabs was chosen, because disinfection residues were neutralised by pre-impregnation and the swabs allowed sweeping over large surfaces. Furthermore the pre-packed sterile kits were easy to handle for the sampler and the laboratory received the samples ready for inoculation in a stomacher bag. Successful detection of MRSA has been demonstrated using such cloth swabs in environmental sampling at pig slaughterhouses (Gilbert et al., 2012). The swabs have also been shown to be effective in environmental sampling of enterococci in Swedish broiler production (Nilsson et al., 2009).

Lastly, the swabs were cultured using MH containing 6.5% NaCl, followed by selective enrichment in TSB containing 4% NaCl, aztreonam and cefoxitin, similar to the procedure described in an MRSA screening of dust in pig holdings within the European Union (Anonymous, 2007). In a later study, comparisons were made between the same MH as we used, followed by
selective enrichment in PMB with aztreonam and ceftizoxime and direct selective enrichment in TSB containing 4% NaCl, aztreonam and ceftizoxime for environmental MRSA detection in an equine hospital (van Duijkeren et al., 2010). The use of two broths gave higher numbers of positive samples. Our culture method was a mix of the two, with the exceptions that the concentration of aztreonam was 75 mg/L, not 50 mg/L, and cefoxitin was used instead of ceftizoxime. Direct comparison is difficult, as details differ between studies.

### 3.6 Infection prevention and control

In order to approach the topic prevention and control of nosocomial infections as MRSA in equine hospitals, outbreak and intervention studies were applied (Papers I, III and IV). We analysed interventions to curb an outbreak, established baseline data on IC operations and compliance rates with IC procedures in relation to an intervention. We also tested methods that are used in human health care to measure compliance in equine hospitals.

#### 3.6.1 The Orion statement

Outbreak studies are by nature non-randomised, while intervention studies can be either controlled or non-randomised (or quasi-experimental). Here the intervention study was non-randomised and subjected to uncontrollable factors. The ORION statement (Outbreak Reports and Intervention Studies of Nosocomial Infections) used in human hospital epidemiology is professionally agreed advice for such studies (Stone et al., 2007). This statement was employed in the equine hospitals studied here (Paper III and IV). The ORION statement emphasises the need for correct and detailed description and definition of study design, material and methods. Possible biases and confounders should also be addressed, as always, for a scientific understanding and evaluation of such studies. It was challenging to follow the guidelines. However, factors such as missing data, if due to inefficient IC operation, were also revealed knowledge.

#### 3.6.2 Intervention and measure of compliance

Evaluation of the intervention effect was not possible with the study design used in Paper IV. However, comparison of the measures decided upon for determining compliance over time was possible. The observed intra- and inter-hospital trends over time were able to generate questions and hypotheses. The methods used to measure compliance, direct observation of compliance and indirect purchase figures are also used for audit, in particular hand hygiene.
compliance, in human health care (Boyce, 2011; WHO, 2009; Haas & Larson, 2007). Logically, both methods would be valid also in equine hospitals.

**Observations of compliance**

Observation is considered the ‘gold standard’ for determining hand hygiene compliance in human health care (WHO, 2009). The advantage with observations is the detailed information gathered, e.g. on whether all elements of hand hygiene procedures are being accurately applied (Sax et al., 2007). The disadvantages are that it is time-consuming, costly and provides a low percentage of the total hand hygiene occasions executed. Other aspects to consider are the definition of compliance versus non-compliance and whether the observations are applied around-the-clock (Boyce, 2011). The risk of altered behaviour during observation (the Hawthorne effect) (Eckmanns et al., 2006) was avoided by using an appointed observer from the staff, who naturally merged in. On the other hand, none of the observers in our study was validated, a requirement in the current ‘gold standard’ (WHO, 2009). The possibility of measure or recall bias must also be considered. However, a fairly reliable intra-hospital trend would likely appear by the use of one observer at each hospital throughout the study. The observations had to be adapted to match the prevailing IC operations in each of the study hospitals.

Self-assessment could have been an option as it is less laborious, but overestimation of compliance is a problem (Boyce, 2011).

**Purchase data**

The pure data on purchase figures and total number of patients should not be subject to selection or recall bias. Still, in cases of wastage or used for purposes other than intended bias will occur (Gould et al., 2011). The data are easier to gather and less time consuming than direct observations. The calculation of purchase figures per patient might create a bias, as the length of stay of patients was not recorded. A more reliable figure would be purchase per patient day, used in human health care (Sroka et al., 2010; Pittet et al., 2000). For comparison, the hospitals studied here were asked to report data on patient days. Retrospective figures for the purchase figures and numbers of patients were gathered for a period (9-12 months) prior to the actual study as well as the pre- and post-intervention figures. The pre-study figures were used as the control when evaluating the pre- and post-intervention data.
3.7 Questionnaire

A questionnaire constructed by the author to this thesis was applied in Paper IV. It was a convenient way to collect data, but could be subject to low response rate. How questions are asked influences the answers and could be adjusted by control questions. This was not considered here, as the questionnaire used was very short, with two closed and two open questions to get baseline knowledge of opinions and experience of IC in the study hospitals to add to other information on the topic.

3.8 Statistics

Descriptive statistics, e.g. median carriage time in Paper II and individual result presentations were used in all studies.

In Paper II, a case was considered MRSA-positive if any sampling site tested positive. The total number of positive occasions for a sampled site was compared with the total number of positive sampling occasions in order to calculate a relative sensitivity and confidence interval (CI) in detecting MRSA for each site.

Descriptive statistics on purchase per patient and patient day were calculated in Paper IV. In this study Fisher’s exact test was also used for calculating the significance of changes in figures between the measured time periods.
4 Results and discussion

4.1 Outbreak epidemiology

During the summer of 2008 six horses at an equine hospital were infected with MRSA CC398, spa-type, t011 (Paper I). PFGE analyses of isolates from the screening and outbreak cases revealed one pulsotype. This, plus the spatial and temporal relationship of the infected horses, confirmed an outbreak. The index case of the outbreak was not identified. However more important was to defining the hub for infections, the surgery unit, as being central to curb the outbreak. An exogenous source, i.e. not the horse itself, must be expected for some of the cases and the findings point out the importance of efficient IC operations.

4.1.1 Occurrence of MRSA spa-type t011

MRSA of the same pulsotype as the outbreak isolates had been found once in Sweden prior to the outbreak. In a horse in a screening survey in 2007 (SVARM, 2008). The same strain was also detected in the few sporadically infected cases (n=4) up to almost two years after the outbreak. One of the sporadic cases, just over a year after the outbreak, was connected to another hospital in the area (Paper I). The MRSA isolate from this horse had a slightly different pulsotype as shown by PFGE (Figure 2 in Paper I). It was still interpreted to be of the same origin as the outbreak isolates (Tenover et al., 1995), but exemplifies genetic changes in bacterial strains. Previous MRSA infections and carriage in horses in other European countries have also been associated with spa-type t011, providing evidence of the emergence of this MRSA type in horses. For example, in the Veterinary University of Vienna, nine cases have been recorded (Cuny et al., 2008). In outbreaks in a Dutch equine hospital, t011 was the dominant spa-type (van Duijkeren et al., 2010). Carriage of MRSA t011 was also revealed in screening of horses on admission
to a Belgian clinic (Van den Eede et al., 2009). The wide spread of MRSA in horses is further reminder to equine hospitals to review their IC status.

In Sweden, one other finding of spa-type t011 in animals has been reported. Four screening surveys (2006 to 2011) of pigs and pig farm environments in Sweden revealed one MRSA-positive sample, and this was of spa-type t011 (SVARM, 2012). MRSA CC398, that spa-type t011 belong to, has not been detected in cattle or pets in Sweden (SVARM, 2012).

Horses and professionals handling or treating horses travel a lot and a possible explanation for the appearance in Sweden could be spread from abroad. Epidemiological comparison of spa-type t011 isolates from different sources and species, e.g. by PFGE, would be interesting.

4.1.2 Outcome

None of the infected horses was treated with antimicrobials after MRSA diagnosis. The infection cleared in all cases available for evaluation, which were nine out of 10. All nine had superficial skin infections except for one with an extensor tendon rupture, visible bone but intact periosteum. The observation that infections cleared was encouraging, or as one clinician expressed it “the most fantastic experience of the outbreak”. The results emphasise the need for research to improve the evidence base for wound treatment in general and to define (in guidelines) when, if ever, antimicrobials are needed in such cases.

4.1.3 Zoonotic aspect

The zoonotic risk for staff at the hospital had to be considered, as during an outbreak of MRSA the risk of becoming contaminated and possibly colonised is increased. However, infection in humans with MRSA CC398 is considered uncommon (Graveland et al., 2011a; van Cleef et al., 2011). Studies have reported high figures of positive MRSA testing in veterinary personnel, but these studies mostly concern another type, MRSA CC8 (Anderson et al., 2008; Hanselman et al., 2006; Moodley et al., 2006; Wulf et al., 2006; Weese et al., 2005a). MRSA CC398 is common in livestock, and the carriage rate of this clone in professionals working with or handling these animals is known to be higher than in the average population (Graveland et al., 2011a; Moodley et al., 2008). In Sweden, 10 spa-type t011 isolates of human origin have been detected, two in subjects with known contact with horses or other domestic animals (2006 to 2011) (SWEDRES, 2012).
4.2 Post-infection carriage

The nostrils were the most sensitive sampling site, testing positive on 10 of 11 positive occasions or 0.91 (95% CI: 0.59–1.00). Other sites had relative positive values of 0-0.09. All post-infected cases became test-negative over time (median 143 days), but individual differences were noted. Furthermore, the results indicate that our definition of decolonisation, two consecutive negative samples, was accurate, as after this had been fulfilled all cases stayed negative.

![Image](https://via.placeholder.com/150)

*Figure 1. Trying to reach the sampling site in a cute little patient. Photo K. Bergström*

4.2.1 Sampling sites

The fact that the nostrils were a sensitive site for MRSA screening is in agreement with another study. Hospitalised non-infected horses were sampled in nine different body sites, and nostrils were found to be the most sensitive (83%) (Van den Eede et al., 2012). Moreover, a recent study showed that the optimum site of three tested within the nostrils was the vestibulum (Van den Eede et al., 2013), which is approximately the site we chose, i.e. on the border of skin and mucosa. Considering the results of our studies and those of Van
den Eede et al. (2012 and 2013), we postulate that the nostrils are the optimal first choice to sample for MRSA screening in horses, preferably in the anterior part of the nostrils, although that needs to be confirmed. The previously infected site tested positive just once in one case, on the first sampling in the follow-up. This indicates that healed wounds pose a low risk of dissemination.

4.2.2 MRSA carriage in horses

Six horses in close contact with post-infected cases were sampled in the nostrils on one to two occasions and all tested negative (unpublished data, Paper II). This close contact involved two mares with post-infected foals and paddock mates or housed next to a post-infected case. Due to the small number of cases this result was excluded from Paper II. One MRSA-negative contact was a mare whose foal was repeatedly MRSA positive while still nursing. This raises the question of whether individual genetic differences could be involved in carriage, as has been suggested for carriage of *S. aureus* in humans, where: "persistent carriage depends, at least in part, on an adequate biological match between human host and colonizing *S. aureus* strain" (Nouwen et al., 2004). Higher levels of certain serum antibodies to *S. aureus* in persistent human carriers compared with intermittent carriers and non-carriers indicate that there could be genetic differences affecting the host’s interaction with certain strains (van Belkum et al., 2009). Such ideas were also aired in a study where horses continued to test negative for MRSA regardless of high prevalence of MRSA, while others tested positive on several occasions (van Duijkeren et al., 2010).

MRSA carriage in horses needs to be studied in greater detail. Most studies have tested horses once, in the nose. Horses testing positive once could be transiently contaminated, persistent carriers or colonised. In human medicine the word colonisation is avoided by the use of *S. aureus* carrier categories: (i) persistent carrier, i.e. long-term of mostly one type of *S. aureus*; (ii) intermittent carrier (irregular and change of strains); and (iii) non carrier (Kluytmans et al., 1997). Persistence of MRSA in humans carries an increased risk of becoming infected and is therefore an important classification (van Belkum et al., 2009; Nouwen et al., 2004). Testing MRSA positive on admission to hospital was shown a risk factor also for horse to get infected (Weese et al., 2006). Persistence of LA-MRSA (CC398) in humans seems dependent on the intensity of the animal contact. In the absence of animal contact, the presence decreases rapidly (Graveland et al., 2011b). A more accurate term for this might be ‘constantly re-contaminated’.

Based on Paper II and the equine and human studies presented to date, two questions arise: Can horses be persistent carriers? If so, what are the features of this and does it differ between MRSA strains?
4.3 Environmental detection in prevention and control of MRSA

Potential routes of transmission of the MRSA strain within the outbreak hospital were demonstrated by the environmental sampling in Paper III. The positive sites were scattered over the hospital (Table 2 and Figure 1 in Paper III) and included areas where both humans only had access and areas where horses were kept. Positive environmental samples were incidentally found also where horses had been moved within the hospital. Moreover, the MRSA findings were made after cleaning and disinfection carried out as prescribed by the hospital’s IC procedures.

4.3.1 Contamination

The most important finding of the environmental screening was inadequate hand hygiene. Hands are considered a key route of MRSA transmission within veterinary hospitals (Leonard & Markey, 2008) and good hand hygiene is deemed the single most important preventive measure for nosocomial infections in humans (WHO, 2009; Boyce & Pittet, 2002). The fact that MRSA was found where horses with infected wounds were kept is maybe less surprising, but suggests that infected horses should, if possible, be kept isolated in one place. Note, however, that non-exercise in the case of isolation might lead to colic and require preventive measures.

4.3.2 Cleaning and disinfection

The findings after cleaning etc. indicate that cleaning procedures as well should be subject to regular review. Efficiency of cleaning and disinfection against transmission of pathogens has not been investigated as single intervention in healthcare (Dancer, 2011; Rutala & Weber, 1999). Though, a recent study showed a decreased risk of new patients acquiring multidrug-resistant bacteria when patient rooms were decontaminated by hydrogen peroxide vapour compared with standard cleaning (Passaretti et al., 2013). However, for MRSA the reduction was not significant. Direct misting of peroxygen disinfectant for environmental decontamination in a large animal hospital resulted in almost total reductions in S. aureus and S. Typhimurium (Patterson et al., 2005). Unfortunately the contaminated material tested was of polyester, not a surface typically found in large animal hospitals. Misting has been used for real-life disinfection in a salmonella outbreak in a large animal hospital but the effect of this misting was not evaluable due to other parallel interventions (Steneroden et al., 2010). Studies of real-life situations are needed, as this is where the battle against nosocomial infections takes place, but the studies cited above show the complexity of proving the effect of interventions. The potential risk of nosocomial MRSA spread in a
contaminated environment and the effects of disinfection need to be further studied. A cautious approach seems wise until more evidence is available.

4.4 Outbreak interventions, infection prevention and control

The interventions introduced due to the outbreak (Paper I and III) focused on interruption of indirect contact and spread of MRSA between horses via staff, equipment and environment. Supportive management, establishment of an IC committee with an executive working group and the achievements of staff were important in building an IC operation. The estimated cost of the studied outbreak was approximately 120,000 Euros (170,000 US$) or 20,000 Euros per case detected (taking the average exchange rate during the intervention period). The total course of interventions is described in Paper III (Table 1 and Figure 1). Important features are discussed below, divided into detection, response, intervention and implementation, and are applicable to IC operations in general.

4.4.1 Detection

There was a lag of over a month between surgery and detection of MRSA-infected cases in the outbreak studied. An earlier alert might have prevented the second cluster of infections. The effect on time to detection of factors such as late sampling, negative culture when sampled, sample delivery time and Sundays/holidays off at the laboratory is shown in Table 1 (based on data from Paper I). It was shown that a long lag phase was very costly in a salmonella outbreak at a large animal hospital in the USA, resulting in a total of 4.2 million US$ in costs (Dallap Schaer et al., 2010). The importance of early detection has also been pointed out by others (van der Zee et al., 2013; Petrosillo et al., 2005). The suffering of individual animals and the zoonotic risk from late detection must also be considered.

Five of the six detected cases in the Swedish outbreak were either readmitted to hospital or sampled in the home stable. Late detection of cases could have been affected by the fact that the hospital had no control over horses at home. In fact one case was detected when owners of horses that had undergone surgery during the outbreak were traced by phone. Awareness and sampling outside the hospital are also important for early detection.

The time for culture, 4-10 days, was taken from the time when the final answer left the laboratory (Table 1). However, preliminary reports are customarily issued by the laboratory and suspicion of MRSA might have preceded the final answer. The turn-around time of culture depends on culture method, skills, priorities and opening hours at laboratories. The use of in-house
laboratories in veterinary hospitals has been suggested as a way to cut the delivery lag. However, capacity and updated knowledge could be difficult to maintain, leading to insufficient quality of the analysis and delays if isolates need to be sent for confirmation. Furthermore false positive MRSA detection could be as devastating as false negative.

Table 1. Outbreak of surgical site MRSA infection in an equine hospital

<table>
<thead>
<tr>
<th>Case</th>
<th>Surgery</th>
<th>Sampling</th>
<th>Culture</th>
<th>Answer</th>
<th>Culture sum</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 May</td>
<td>11</td>
<td>12</td>
<td>22</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>26 May</td>
<td>42</td>
<td>46</td>
<td>52</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>6 June</td>
<td>21</td>
<td>24</td>
<td>28</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>2 July</td>
<td>5 and 26</td>
<td>7 and 29</td>
<td>9 and 35</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>4 July</td>
<td>20</td>
<td>20</td>
<td>26</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>7 July</td>
<td>8 and 9</td>
<td>8 and 10</td>
<td>15 and 18</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

1) Date of surgery; 2) Days from surgery to bacteriological sampling; 3) Days from surgery to start of culture; 4) Total time in days from surgery to laboratory report; 5) Laboratory turn-around time; 6) Chronological order of MRSA diagnosis; 7) Negative in first, positive in second sample.

4.4.2 Response

The prompt suspension of elective surgery was an important initial response to the outbreak and the first intervention. ‘Wait and see’ could have resulted in more cases. Case 6 (Table 1), an emergency requiring surgery three days after the suspension of elective surgery exemplifies this. Emergency surgery was not suspended for animal welfare reasons. The decision on suspension of elective surgery also released staff resources from the surgery unit to clean and disinfect. A clearly documented delegation of responsibility and authority to act on alarms to avoid delays must be set up by the managers. Training staff and increasing clinical awareness are important in order to be able to respond to alarms (Ippolito et al., 2006; Petrosillo et al., 2005).

4.4.3 Interventions

The practical intervention phase was the most extensive and long-lasting in the Swedish outbreak, as described in detail (Table 1 in Paper III). The easy part was to write policy documents on directly transferrable procedures from human health care, such as basic personal hygiene (hand sanitising, no rings or wrist watches, short nails, disposable gloves in contact with potential contagious secretions, etc.). The basic hygiene procedures were important documents as hands is considered an MRSA transmission route in animal hospitals (Leonard & Markey, 2008). A review also found a strong association
between increased use of alcohol hand rub and lowering of human MRSA rates (Sroka et al., 2010).

**Dress regulation**

The dress regulations, with short sleeves in contact with patients, encountered some problems because of lower temperatures in the hospital during winter. The problem was resolved by use of fleece gilets (washable at 60 °C) and designated jackets for outdoor use.

**Hospital interior and equipment**

When it came to equipment and interior surfaces, it was more problematic to develop efficient and applicable procedures. Concrete flooring and porous wooden walls between stalls, which can be found in most equine hospitals, proved difficult to sanitise and therefore need to be replaced with innovative materials.

MRSA has also shown good survival in dust (Oie & Kamiya, 1996). A significant association between high activity levels of humans and high airborne presence of *Staphylococcus* spp. was shown in a veterinary teaching hospital (Lutz et al., 2013). The role of horse hair coat as potent MRSA carrier is not studied though.

Another general challenge is the surgery unit. Moving the anesthetised heavy animal to and from the operating table includes several risk elements in the spread of infective agents, e.g. reusable mattresses, tarpaulins, etc. Moreover, recovery stalls have to include some type of non-slip padded surfaces, as horses awakening from general anaesthesia may be unsteady on their legs and if they fall the risk of injuries from unpadded surfaces is obvious. However, the padding continuously gets scratches that are difficult to clean and where bacteria and organic material can hide.

**Footwear**

Footwear hygiene was discussed and should be applied by regular cleaning of boots and shoes. The use of footmats, footbaths or rubber overboots to reduce dissemination of infective disease is a controversial topic. These measures have been proven to be effective in broiler production in reduction of *Campylobacter* spp. infections (Gibbens et al., 2001; Evans & Sayers, 2000). In contrast, in equine hospital environments they are reported to have no influence on the bacterial load on floors (Stockton et al., 2006). If footmats or footbaths are used, it is likely that how they are used is what matters. Dirty shoes or boots will not be disinfected by quick dips in footbaths. Wrongly used, they might instead give a false sense of security and less cautious
behaviour. The significance of footwear as a risk factor for MRSA dissemination is not actually known and has to be evaluated.

**Sampling of horses**

Because of the MRSA outbreak (Paper I) it was stated that all horses with infected wounds, both surgical and traumatic, should be sampled for monitoring purposes in the outbreak hospital. In the salmonella outbreak mentioned above (Dallap Schaer et al., 2010) the reliance on passive and not comprehensive patient monitoring was one cause of delayed detection. Systematic collection of culture data, allowing assessment of current status, should preferably also be implemented. Infection monitoring includes providing written information to horse owners with thoroughly described infection signs and emphasising the need to contact the hospital if such signs appear after discharge. In an outbreak situation such as that studied in Papers I and III, a procedure of actively tracing the owners of discharged horses seems wise to detect hidden cases. Active tracing is also important for the safety of the horses and humans in the home stable.

The outbreak studied here also started a discussion at equine hospitals about MRSA screening of horses on admission, for early detection of MRSA. Screening of all horses by nostril sample on admission, weekly and at discharge was implemented in a Veterinary Teaching Hospital (Weese et al., 2006). Evaluation of the program has shown that horses testing positive on admission are more likely to suffer from MRSA infection than those testing negative (concerns CMRSA-2 and CMRSA-5, equal to CC5 and CC8 respectively). In Sweden, general screening on admission seems less advisable in the current circumstances. If such testing on admission is considered, a cost-benefit analysis could be needed. Testing requires isolation of the horses until the test result arrives. Studies in humans have shown that isolation of a patient when the positive result is obtained does not reduce transmission to other patients if basic precautions are not consistently taken (Cepeda et al., 2005). A recent Dutch study of human hospitals with a baseline prevalence of 20% MRSA in intensive care units (ICU) and 5% as a whole compared the cost-benefit of four screening and isolation strategies against doing nothing. The most cost-effective option was to screen and isolate known carriers and ICU patients. Testing of defined risk patients could also be considered in equine hospitals, but requires knowledge of factors associated with carriage of MRSA in horses. There are a limited number of studies on risk factors to date (Anderson et al., 2008; Weese & Lefebvre, 2007; Weese et al., 2006). Apparent risk factors would be horses coming from known high prevalence areas, hospitalised in such areas or connected to outbreaks. Other risk factors
are summarised in section 3.3. A strategy could be to handle all horses or defined risk patients with high precautions. False negative testing does occur, reported even with PCR screening in humans (Andersen et al., 2010).

The challenges that the differing equine hospital environment conveys compared to human health care need to be dealt with. Some could be resolved by renovations or innovations or on-the-spot cleaning, while others need research, e.g. engineering innovations in terms of surface material.

4.4.4 Implementation

Some of the procedures described in Paper III concerned all staff, i.e. basic hygiene instruction sheets had to be actively read and signed by all (also students). The procedures concerning some staff were available to all, but mandatory only to whom they concerned. Education and information seminars on MRSA and IC operations in general completed the training. This was to drastically decrease insufficient knowledge as a cause of non-compliance.

Implementation is a comprehensive task for all health care professionals, including staff in equine hospitals. Implementation science, or “knowledge to practice”, is a relatively new and complex field of inquiry (Graham & Tetroe, 2007). The core message is expressed very aptly in the abstract of sepsis bundle study: “Knowledge translation is the science of accelerating the transfer of knowledge to practice by understanding and creatively addressing the barriers that prevent adoption of new professional standards” (Stoneking et al., 2011). Furthermore, the influence of habits, attitudes and social norms has to be considered and needs to be studied using multidisciplinary research methods to obtain the knowledge and insights needed to generate successful implementation tools (Sax et al., 2013).

Awareness is a key phase in the implementation process in the journey from “knowledge to practice” (Graham & Tetroe, 2007). One example of awareness in these studies was clearing of infections without antimicrobial treatment. An example of unawareness was a member of staff, took a mobile phone out inside an isolation stall to switch it off. The message about not talking on mobile phones inside isolation was received. However, the deeper understanding of avoiding contact with items that are used also outside isolation was lacking. Such is an important implementation issue to deal with.

The outbreak studies (Papers I and III) raised questions regarding the extent to which the implemented IC procedures were complied with in equine hospitals in general, and led to examination of IC procedures in Swedish equine hospitals to gather baseline knowledge on the topic (Paper IV).
4.5 Intervention and surveillance of compliance

Purchase figures relating to hand sanitisers and disposable gloves and number of patients were reported according to plan by the hospitals in Paper IV. The observations of compliance were unevenly reported, i.e. reporting prior to the intervention was missing in some cases. The observation measure was therefore less valuable than the calculated purchase per patient figures. The level and type of observations varied between the hospitals, as their current IC procedures differed. The questionnaire, answered by approximately half the staff in the hospitals, revealed three main themes of barriers to compliance. Practical barriers, such as insufficient supply of hygiene products, were most commonly reported. The other two were insufficient knowledge of IC operations and large workload. Lack of available hand rubs and high activity or workload have also been correlated to lower compliance in studies in human hospitals (Rupp et al., 2008; Traore et al., 2007). The opinions expressed in the questionnaire were all important if the management is interested in improving IC compliance. A questionnaire could be repeated to follow-up improvements in the IC systems.

In the following, the results are discussed hospital by hospital and then general features are summarised.

4.5.1 Hospital A

The compliance with dress regulations and personal appearance (no rings, wrist watches, short nails, etc.) reported during both the pre- and post-intervention period was excellent. The hand hygiene procedure was divided into two sets of observations. Hand washing (with soap and water) was also excellent both pre- and post-intervention, while hand sanitising was less well complied with. The purchase figures reported were markedly higher prior to the study than within the actual intervention period studied (Table 2). This hospital was the outbreak hospital described in Papers I and III. It is likely that increased compliance was induced by the outbreak rather than by our intervention. To test this argument, purchase figures prior to the outbreak would have been required for comparison, but was not part of this study. Nonetheless, the reduced purchases during the study period suggest a decrease in compliance some time after the outbreak. The implementation seemed to have failed to maintain compliance.
Table 2. Purchases monitored at three equine hospitals during an intervention study (source: Paper IV)

<table>
<thead>
<tr>
<th>Purchase figures on hand hygiene products per patient (per patient day)</th>
<th>Pre-study $^{31}$</th>
<th>Pre-intervention $^{21}$</th>
<th>Post-intervention $^{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospital A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months of recording</td>
<td>12</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Hand sanitisers in mL</td>
<td>82</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Pairs of gloves</td>
<td>33</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td><strong>Hospital B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months of recording</td>
<td>9</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Hand sanitisers in mL</td>
<td>3 (2)</td>
<td>6 (4)</td>
<td>8 (5)</td>
</tr>
<tr>
<td>Pairs of gloves</td>
<td>7 (5)</td>
<td>13 (8)</td>
<td>13 (9)</td>
</tr>
<tr>
<td><strong>Hospital C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months of recording</td>
<td>12</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Hand sanitisers in mL</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Pairs of gloves</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

1) Retrospective figures prior to the actual start of the study
2) Figures prior to the intervention, an education about MRSA and hygiene procedures
3) Figures after the intervention

4.5.2 Hospital B

Hospital B reported an increase in purchase figures per patient prior to our intervention. The reported data made it possible to calculate the figures also per patient day. However, calculations on per patient or per patient day gave similar information about the trends over time (Table 2). This hospital had high compliance with hand hygiene, on average 93% based on 487 observations (Table 3 in Paper IV). However, observations were only made after the intervention. Compliance with the dress regulations and personal appearance procedures was also excellent, but again reported only post-intervention. The hospital management also reported that disposable gloves were used in almost all operations, even walking horses to the paddock. A speculative explanation for this hospital’s purchase trend (Table 2) prior to our intervention could be a subconscious ‘gearing up’ during preparations for the study. Its managers had shown a proactive interest in IC operations.

4.5.3 Hospital C

The reported observations on glove procedures showed significantly increased compliance after the intervention (Fisher’s exact test p=0.015). The trend noted for the indirect figures on glove purchases also increased. Such associations
have been shown by others (Eckmanns et al., 2006) but also the opposite, i.e. no association (Marra et al., 2010). This hospital had the least developed IC system prior to the study and no written documentation. It seems likely that the hospital was affected by the intervention or the impact of the whole study (which was an intervention as well).

4.5.4 General features

Only the trends and not the actual numbers in Table 2 should be compared between hospitals, as their conditions differed. For example, hospital A had many more employees compared with the other two hospitals, and also students and teachers. Furthermore we cannot know if products were used to other than intended.

Trends

The trends differed between the hospitals in relation to the intervention (Table 2). This could of course be due to many reasons, but one question raised was “What motivates compliance?”, as only hospital C showed an increase in measured compliance after our intervention. What impact had other uncontrollable factors on compliance? For example the impact of an outbreak compared with that of dedicated management, etc. More studies are required to provide answers, which might be useful in creating efficient triggers as implementation tools.

Implementation

Compliance with the dress regulations and personal appearance was excellent overall. This suggests that the implementation for these procedures had succeeded. Hand hygiene was poorer and some findings indicated that this was more complex than just poor compliance. The excellent compliance with hand washing but poorer compliance with hand sanitation procedures in hospital A indicates that hand washing was compiled at the expense of hand sanitising, for some reason. Similar results were noticed in a study of 18 small animal hospitals, where hand washing with water and soap was far more common (85%) than use of alcohol-based hand sanitiser (12%) (Nakamura et al., 2012). That the hands get dirty when handling animals makes it natural to use soap and water. According to the hand hygiene procedure in hospital A this was also the correct first step in hand hygiene. The policy was: In the case of visible dirt, washing with soap and disinfecting hands between patients and/or different procedures; if no visible dirt only disinfection between patients or procedures. What we called poor compliance in Paper IV could have been misinterpretation or failure to implement the total procedures, i.e. hand
wearing was interpreted as enough. If that were the case, efforts should concentrate on thorough practical training on how hand hygiene should be practised. Education and training to prevent gloves being used instead of hand hygiene might also be needed. Determining whether hand washing is considered equal to hand sanitising requires complementary information, as interviews with appointed observers and some of the staff at the hospitals, plus more observations.

Comparative studies on compliance with hand hygiene procedures in animal hospitals are few. In addition to the study by Nakamura et al. (2012) mentioned above, an intervention study in small animal practices showed baseline compliance with hand hygiene procedures pre-education (20.6%; 117/568), while post-education the rate was significantly higher (41.7%; 78/187) (Shea & Shaw, 2012). In a systematic review of studies on hand hygiene compliance in human hospital care the overall median was similar to the veterinary study, 40% (Erasmus et al., 2010). A questionnaire study of IC policies covering hospitals in 32 European countries reported 33% unacceptable non-compliance with hand hygiene procedures (Struelens et al., 2006). Poor compliance with hand hygiene procedures in human health care has also been reported by others.

**Suitable monitoring tools**

The hospitals had no problems in reporting purchase and patient figures. Those figures were readily and easily available and can be used to monitor a hospital’s trends over time. This is also a well-known and widely used method in human health care (Eckmanns et al., 2006). In Paper IV a volume of 1.7 mL was suggested for calculation of number of sanitising occasions (McGuickin et al., 1999). The amount is less than the 2-4 mL for hand sanitising recommended by The Swedish Institute for Communicable Disease Control (SMI, 2012). Another, less exact amount is a palmful of the product in a cupped hand (WHO, 2009). However, for calculation of hand sanitising occasions in a setting, it seems most important to define a reasonable denominator and keep to that figure for comparisons over time. A more exact and comprehensive, but also more costly, method is use of an electronic counting devices inside a dispenser, with measured data uploaded to a computer, i.e. direct feedback (Chen et al., 2013; Boyce et al., 2009). The electronic method also provides frequency rates for hand hygiene events.

Observations of procedures were unevenly reported, most likely because it was laborious. Therefore this approach seems less useful for continuous monitoring of compliance in equine hospitals. Spot observations could be used
to obtain more detailed information on the accuracy in performance of a procedure.
5 Conclusions

In relation to prevention and control of MRSA in equine hospitals, the following conclusions can be drawn from the results presented in this thesis:

- The first detected outbreak of MRSA in horses in Sweden comprised surgical site infections caused by a commonly detected MRSA type in horses and livestock in Europe, CC398,spa-type t011. All superficial infections healed without antimicrobial treatment.

- The most reliable sampling site for screening of MRSA was the nostrils. All horses studied tested negative for MRSA over time post-infection, but individual differences in time were noted. The proposed definition for MRSA decolonisation, two consecutive negative samples, was corroborated by the results.

- Environmental MRSA screening was shown to be useful as a tool in prevention and control of MRSA, in the implementation of basic hygiene and for identification of surfaces and items difficult to sanitise in need of renovation or replacing.

- Rapid detection and response combined with multidisciplinary cooperation is the key to successful outbreak control. Infection control procedures used in human health care were mainly applicable, but existing differences between equine and human settings also required adapted solutions. The outbreak had a substantial financial impact for the hospital, of approximately 200,000 Euros.

- An indirect measure of compliance, purchases of hygiene products per patient, was applicable in the equine hospitals studied. Observations of compliance were less readily applicable. Excellent compliance with dress
regulations and personal appearance by staff was observed at all hospitals studied. Compliance with hand hygiene was poorer. Lack of readily accessible hygiene products, insufficient knowledge and high work load were identified as potential barriers to compliance.

5.1 Concluding remarks

The thesis demonstrates the importance of a comprehensive, multidisciplinary approach to prevent and control MRSA in equine hospitals. The elements required range from epidemiological knowledge of MRSA in horses over species-specific adapted IC procedures to implementation and compliance.

When a functioning IC operation is the goal, initial documentation and implementation of procedures must be followed by a running, administrative phase. The administrative phase requires resources, time and education of staff. Furthermore, a chain of responsibility and authority to act on alarms and designated staff to handle the system has to be defined. Human power is required to support the operation after written procedures have been drawn up, as documents will not do the job. The managers of equine hospitals are responsible for the safety of patients and staff, and they also have the power to allocate resources and set the direction taken.

5.2 Future perspectives

As could be understood by the thesis prevention and control in equine hospitals has not been target for major research. The topics discussed in the thesis generated additional research questions and a vision.

- MRSA carriage in horses has to be defined, as contaminated horses testing positive are of less risk than e.g. a persistent carrier. A standardised sampling and culture method should preferable be decided in testing for carriage.

- A complex topic is the performance of different protocols in environmental MRSA detection. Both sampling devices and culture methods needs to be evaluated.

- Engineering innovations for surface materials that are easy to sanitise but still suitable for the horses are required.
> Strict compliance with hand hygiene procedures and disposable gloves in equine hospitals seems complex and could benefit from deeper studies.

> Implementation of interventions is a grey area, not studied in veterinary hospitals, but an important topic according to the results and discussion of implementation and compliance.

> Involvement of multidisciplinary study methods and lateral thinking is necessary to identify the best IC practice for equine hospitals.

> Finally a vision. Trainee opportunities for veterinarians to become ‘Veterinary infection prevention and control diplomats’. In Europe, to my knowledge, there is no such training programme. However, since 2009 the Swedish Veterinary Nursing programme has included infection prevention and control as one of three main specialist areas.
Förebygga och kontrollera meticillinresistent *Staphylococcus aureus* på hästsjukhus i Sverige

**Inledning**


**MRSA utbrott**

Långtidsprovtagning av hästar efter infektion

Hantering av ett utbrott
I avhandlingens tredje studie redogörs för åtgärder som utfördes på utbrottssjukhuset för att förhindra att hästar smittades av MRSA. Samverkan mellan sjukhuset, vårdförmånsexpertis från humanmedicin och myndigheter bidrog till utvecklingen av ett infektionskontrollprogram. Miljön på ett hästsjukhus medför dock utmaningar och gjorde att en anpassning av vissa hygienrutiner krävdes. Fortsatta studier om hur miljön kan anpassas, t.ex. ytmaterial som passar för hästar och är lätt att desinficera, behövs för att vidareutveckla infektionskontroll inom hästsjukvård. Provtagning av miljön för att påvisa MRSA gjordes också. Positiva prov från tagställen där endast människor hade tillträde visade brister i följsamhet till handhygien. Vidare byttes t.ex. krubbor och vattenkoppar som var svåra att rengöra mot sanerbara hinkar eftersom MRSA hittades i prov från sådan inredning. Miljöprovtagning ska dock tolkas med försiktighet, negativa fynd innebär inte att miljön är fri från MRSA.

Vårdhygien på svenska hästsjukhus
**Summering**


Underhåll av ett infektionskontrollprogram kräver fortlöpande arbete med revisioner, utbildning och övervakning. Sjukhusledningar måste stötta genom att tillsätta resurser och genom aktivt engagemang. Införande av infektionsförebyggande och kontrollerande åtgärder är ett självklart ansvar som åligger hästsjukhus då MRSA berör både patientsäkerhet och arbetsmiljö.
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Acknowledgements

As all PhD students I have many to acknowledge. In my case this “heavy” work came rather late in life, but better late than ever??

First Görel, who came one day and said: “Karin, PhD time”! And here we are ready for the dissertation. Thanks for your professional view, your ocean of experience, looking at things from above. “The core, Karin”.

I would also like to give great thanks to...

..Ulrika! We applied for research funding, discussed results, wrote manuscripts, travelled to Guelph, tasted wines, shopped and went to conferences…Working hard, but also having a lot of fun.

..Christina! In a room near to mine, patiently answering more or less clever questions, so bright, full of information and always on time.

..Asa at Uppsala University, who brought human medicine aspects in and taught me that there is a lot to learn in microbiology. I enjoyed the discussions of both study issues and other things.

Coauthors to the Papers, thank you all…

..Annica who ran the laborious PFGE. The photos were high-class.

..Anna for interpret molecular microbiology and support the writing.

..Björn for pensive and thoughtful input to the manuscript.

..Ann for statistics, but also supporting whenever I needed professional advice.

..Ulrika R for highly professional knowledge, experience and support.

..Chris although always busy, time for supportive outbreak information.

..Stefan for the drawing of the environmental sampling and discussions of PhD problems.

Furthermore, thanks to Ma and Kerstin for your skilled invaluable laboratory work.
Thanks also to the horses, horse owners and veterinary practitioners that made collect of cases at all possible. Thanks also to the studied equine hospitals for willingly sharing information, especially to Lena, Marie, Ulrika and Trine.

Great thanks also to the Swedish-Norwegian Foundation for Equine Research for main funding. Thanks also to the Swedish Board of Agriculture for funding the longitudinal sampling of horses.

Many thank to all at the Department of Animal Environment and Health at SLU in Skara for inviting me to the world of research, to Jan, Stefan, Carina. Also thanks to SVA my employer in daily work..for supporting with time and space.

Thanks Gittan, my colleague in the daily work who has supported me and let me focus on my studies full time lately. I will soon be back!

I’m almost finished but … thanks also to all who helped me with small but for me valuable things such as extra proofreading or helping with EndNote for the hilarious SLU template, Stefan, Märit, Oskar, Helena, Micke, Johnny and Rickard at 4444 and the whole staff at DOA and BAKT plus all the ones I have forgot to thank.

Last but definitely not least, thanks to my dear Mats for his support and enormous patience with me over the years. Thanks as well to my two beloved sons, just for being but also, Karl, for having dinner ready when I come home late (… your salmon is delicious). Gustav my son living and study in Lund, supporting my research by reading my papers.

Thank you all!