Bacterial Infections in Dogs with Special Reference to Urinary Tract Infections, Surgical Site Infections and Methicillin-resistant *Staphylococcus pseudintermedius*

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Abstract

An increase in antimicrobial resistance in canine bacterial pathogens, including multidrug-resistance, has been reported worldwide. Increasing antimicrobial resistance is of concern, not only as it complicates therapy in dogs, but also as it is a public health problem when the pathogens are zoonotic, or the location of resistance genes enables transfer between bacteria of animal and human origin.

The overall aims of this thesis were to gain knowledge of bacterial infections in dogs with special reference to urinary tract infections (UTI), surgical site infections (SSI) and their antimicrobial susceptibility patterns, and of carriage of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). The results were intended to aid in choice of antimicrobial treatment of canine UTI and SSI, and in designing recommendations on prevention and control of carriage of MRSP.

First-line antimicrobials were found to be a rational empirical antimicrobial therapy for the studied dog population. In total three percent of detected *Escherichia coli* isolates were resistant to extended-spectrum cephalosporins. Less than 3% of *Staphylococcus pseudintermedius* isolates were methicillin resistant. No methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were found.

Dogs carried MRSP for several months without clinical signs. Systemic treatment for three weeks or longer with antimicrobials to which the bacterium was resistant was associated with prolonged carriage compared to shorter treatment periods. Three of five dogs treated with an antimicrobial to which the MRSP isolates were susceptible remained MRSP carriers. These findings support restricted use of systemic antimicrobial treatment in dogs with possible or confirmed MRSP carriage or infection. The risk of MRSP colonization in dogs living in a household with an MRSP infected dog might be lowered if the clinically infected dog (index dog) becomes MRSP negative. Furthermore, all contact dogs in the family might not carry MRSP continuously during the time the index dog is MRSP positive. The results of the evaluation of five body sites for MRSP carriage screening suggest that simultaneous sampling of pharynx, perineum and the corner of the mouth, as well as wounds when present, should

be recommended. Furthermore, the results suggest that sampling of nostrils is not a priority when screening dogs for MRSP.

Keywords: bacterial infections, dogs, canine, urinary, surgical, antimicrobial resistance methicillin-resistant *Staphylococcus pseudintermedius*

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Contents

List of Publications

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

I **Ulrika Windahl,** Bodil Ström Holst**,** Ann Nyman**,** Ulrika Grönlund and Björn Bengtsson. (2014). Characterisation of bacterial growth and antimicrobial susceptibility patterns in canine urinary tract infections. *BMC Veterinary Research* 10:217.

II **Ulrika Windahl**, Björn Bengtsson, Ann K Nyman and Bodil Ström Holst The distribution of pathogens and their antimicrobial susceptibility patterns among canine surgical wound infections in Sweden in relation to different risk factors. (2015) *Acta Veterinaria Scandinavica* 57:11.

III **Ulrika Windahl**, Elin Reimegård, Bodil Ström Holst, Agneta Egenvall, Liselotte Fernström, Mona Fredriksson, Gunilla Trowald-Wigh and Ulrika Grönlund Andersson. (2012) Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs-a longitudinal study. *BMC Veterinary Research*

IV **Ulrika Windahl,** Joakim Ågren, Bodil Ström Holst, Stefan Börjesson. Colonization of methicillin-resistant *Staphylococcus pseudintermedius* in multi dog households, a longitudinal study using whole genome sequencing. (Manuscript)

Papers I-III are reproduced with the permission of the publishers.

The contribution of Ulrika Windahl to the papers included in this thesis was as follows:

I Design and coordination of the study, participation in the bacterial analyses, interpretation of data, drafting the article, critical revision of the article.

II Design and coordination of the study, interpretation of data, drafting the article, critical revision of the article.

III Design and coordination of the study, interpretation of data, drafting the article, critical revision of the article.

IV Design and coordination of the study, interpretation of data, drafting the article, critical revision of the article.

Abbreviations

BP	Breakpoint
CLSI	Clinical and Laboratory Standards Institute
ESC	Extended-spectrum cephalosporins
HAI	Health care associated infections, also called nosocomial infections
MDR	Multidrug resistance
MIC	Minimum inhibitory concentrations
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSP	Methicillin-resistant *Staphylococcus pseudintermedius*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MSSP	Methicillin-susceptible *Staphylococcus pseudintermedius*
PFGE	Pulsed-field gel electrophoresis
SSI	Surgical site infection
SVA	National Veterinary Institute
UTI	Urinary tract infection
WHO	World health organization

1 Background

Bacterial infections can cause severe morbidity and mortality in dogs, as well as in other animals, and humans. The development of antimicrobials has therefore had a profound positive impact on human and animal health and thereby welfare. Prompt antimicrobial therapy for an infected veterinary patient can make the difference between cure and death or long-term disability. Antimicrobial therapy has also allowed for various medical and surgical advances in the veterinary field, as well as in the human field for more than 50 years (WHO, 2014b, Lloyd, 2010, Weese, 2008a, Guardabassi and Prescott, 2015, Weese *et al*., 2015, WHO, 2014a). In human medicine, a well-documented rapid increase in antimicrobial resistance has been labelled a severe threat to global health by the world health organization (WHO) (WHO, 2014a, WHO, 2014b).

An increase in prevalence of antimicrobial resistance including multidrug resistance (MDR) has during the last two decades been reported in different bacterial species isolated from dogs worldwide (Wieler LH, 2011, Ewers *et al*., 2011, Pellerin *et al*., 1998, Prescott *et al.*, 2002, van Duijkeren *et al*., 2011a, Guardabassi *et al.*, 2004b). Canine antimicrobial resistant bacterial infections can increase morbidity, mortality and prolong hospitalization for the dogs. The infections can also lead to increased treatment cost and emotional strain on the pet owner. In addition to complicating therapy in dogs, increasing antimicrobial resistance in canine pathogens is also of public health concern when the pathogens are zoonotic, or the location of resistance genes enables transfer between bacteria of animal and human origin (Wieler *et al*., 2011a, Ewers *et al*., 2012, Weese, 2008a, Guardabassi *et al*., 2004b).

Several challenges are involved for the attending veterinarian, including assessment of possible health implications for persons involved and which rational antimicrobial treatment strategies to choose. The reports on emerging antimicrobial resistance in important canine pathogens can *per se* put pressure

on clinically active veterinarians to use antimicrobials other than traditional first-line antimicrobials, including agents of utmost importance for human medicine, where they are intended to be used only a last resort choice (Guardabassi *et al*., 2004b, Weese, 2008a, Guardabassi and Prescott, 2015, Wieler LH, 2011, Lloyd, 2010).

Highly resistant bacteria of special concern due to their zoonotic potential include Enterobacteriaceae resistant to extended-spectrum cephalosporins (ESC), and methicillin-resistant *Staphylococcus aureus* (MRSA). Clones of these bacteria, that are resistant to beta-lactam antimicrobials, the most important, and widely used antimicrobial class in both humans and dogs, have emerged as a significant problem in human healthcare worldwide. In addition, MRSA clones and Enterobacteriaceae resistant to other antimicrobial classes as well are an increasing threat in human medicine. The rapid worldwide spread of these pathogens where treatment options often are severely limited make them prime examples of why antimicrobial resistance has been declared a severe threat to global human health (Guardabassi *et al*., 2004b, Weese and van Duijkeren, 2010, Wieler *et al*., 2011b, WHO, 2014a).

Escherichia coli (*E. coli)* and other bacteria belonging to the family Enterobactericeae are ubiquitous colonizers of the gut flora and important pathogens in dogs and other companion animals. Transmission of the antimicrobial resistance genes encoding for ESC resistance can spread between bacteria of the same, or different, species. As the gut flora contains kilograms of bacteria that can exchange such resistance genes, the gut can act as a reservoir for antimicrobial resistance genes (Brolund, 2014, Wieler LH, 2011). An increase in detection of Enterobacteriaceae isolates with transferable genes conferring ESC resistance from dogs is reported worldwide. Clinical infections have also been described (Rubin and Pitout, 2014, Wieler LH, 2011, Guardabassi *et al*., 2004b, Ewers *et al*., 2012). Neither the true prevalence in dogs nor the zoonotic risk is well described (Rubin and Pitout, 2014). A few studies have indicated that exposure to companion animals might be a risk factor for ESBL carriage in humans, e.g. (Leistner *et al*., 2013, Meyer *et al*., 2012, Ewers *et al*., 2012).

Prevalence of Enterobacteriaceae with ESC-resistance is thought to be low in Swedish dogs, as few positive samples are submitted. At the National veterinary Institute, 60 isolates of Enterobacteriaceae with genes conferring ESBL- or AmpC-production were confirmed in dogs and cats in the period 2008 to 2013 (SWEDRES-SVARM, 2013).

MRSA has been labelled a bacterial pathogen of particular international public health concern by the WHO (WHO, 2014a). Humans are natural reservoirs for *S. aureus*, and asymptomatic colonization is far more common

than infection (Chambers, 2001). Canine infection with MRSA has primarily been reported since the late 1990s. Carriage and infection in dogs has since been reported worldwide, including primarily surgical site infections, wound, skin and soft tissue infections (Loeffler and Lloyd, 2010, Weese, 2008c). So far, MRSA seems to be a relatively uncommon pathogen in dogs, but the multidrug resistance and the zoonotic implications makes MRSA an important small animal veterinary pathogen (Loeffler and Lloyd, 2010, Weese, 2008c, Guardabassi, 2013). MRSA strains isolated from dogs and cats have mostly been identical to the MRSA lineages prevalent in human health care in respective geographical region, and carriage appears to be more widespread in areas where MRSA is commonly detected in humans (Loeffler and Lloyd, 2010). Although carriage in dogs has been suggested to be short lived when the individual dog is not continuously exposed to MRSA, the possible role of dogs as reservoirs for MRSA in humans is unclear (Loeffler and Lloyd, 2010). MRSA positive dogs may still play an important role as reservoirs within family households (Guardabassi *et al*., 2004b, Hanselman et al., 2009). Furthermore, higher rates of MRSA carriage in veterinary staff compared to healthy community members have also been reported (Loeffler and Lloyd, 2010). MRSA in dogs could therefore be an occupational risk, as carriers have a higher risk of acquiring an MRSA infection. Conversely, MRSA colonized veterinary personnel might act as sources for MRSA infection in veterinary patients (Weese, 2008a).

Compared to international data, the prevalence of MRSA in Sweden is low both in humans and in animals. MRSA in animals is notifiable since 2008. The first canine MRSA isolate was detected in 2006. Since then less than 30 isolates have been reported according to the Swedish Board of Agriculture (www.jordbruksverket.se) (SVARM, 2010, SWEDRES-SVARM, 2013).

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a multidrug resistant canine pathogen that - albeit with zoonotic potential, as infections and carriage in humans is reported - during the last decade has emerged as a major challenge mainly for small animal veterinarians (van Duijkeren *et al*., 2011a, Frank and Loeffler, 2012).

1.1.1 Health care associated infections

Receiving care in a hospital or in other health care facilities can in itself increase the risk for acquiring antimicrobial resistant infections. Health care associated infections (HAI), also called nosocomial infections, are infections caused by bacteria or other infectious organisms that are acquired by the patient during hospitalization. It was previously thought that nosocomial

infections originated from bacteria that were part of the endogenous flora of the patient, but more recent studies have shown that many HAI are caused by organisms acquired from the hospital environment (Johnson, 2002, Ducel *et al*.). A HAI definition used by WHO is: "*An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility"* (WHO, 2002). Health care-associated infections are one of the leading causes of death in human patients, as well as a significant burden both for the patient and for public health, and as the economic costs are considerable (Ducel *et al*., Reed and Kemmerly, 2009).

The selective effects of antimicrobial drug usage and the spread of resistant clones and resistance genes in a hospital setting leading to increased antimicrobial resistance has for a long time been a well-recognized problem in human medicine. Some patient groups, including surgical patients and immunocompromised individuals, as well as some care environments such as intensive care units are associated with an especially high risk of acquisition of nosocomial infections (WHO, 2002). The risk of dogs acquiring an antimicrobial resistant bacterial infection spread throughout a veterinary healthcare environment (e.g. clinic or hospital) is by now also well recognized in small animal medicine. HAI are increasingly complicated by the emergence of MDR pathogens both in human and small animal veterinary medicine (Wieler *et al*., 2011b, Guardabassi, 2012, Johnson, 2002, Weese and van Duijkeren, 2010, Frank and Loeffler, 2012, Weese, 2012). Enterobacteriaceae resistant to ESC and MRSA are among the most important causes of HAI in human medicine. As colonized and infected patients becomes more prevalent in the community, the risk of community-acquired infections with infections which were initially acquired almost exclusively as HAI infections (HA-MRSA) increases. Presently, some MRSA clones are instead primarily community-associated (CA-MRSA). Such clones can also enter the healthcare environment and infect patients (Chambers, 2001, Lawes *et al*., 2015, Egea *et al*., 2014, Guardabassi, 2012). MRSA is also recognized as a nosocomial pathogen in dogs (Wieler *et al*., 2011b, Vincze *et al*., 2014, Loeffler and Lloyd, 2010).

Sources of infection include the physical environment, bacteria on hands and clothes of personnel, carriage o staphylococci in the nasal passages of the staff, and surgical equipment. Nosocomial pathogens have been shown to persist in the hospital environment in a variety of locations and bacterial colonization of human hospital patients by endemic hospital organisms has been shown to occur in patients within a few days of hospitalization (Johnson,

2002, Glickman, 1981, Hamilton *et al*., 2013, Nelson, 2011, Guardabassi, 2012, Weese and van Duijkeren, 2010, Murphy *et al*., 2010, Ishihara *et al*., 2010, Ducel *et al*.).

The critical role of infection control in veterinary healthcare environments in preventing the spread of multidrug resistant bacteria in small animals has been highlighted (Wieler *et al*., 2011b, Guardabassi, 2012, Johnson, 2002, Weese and van Duijkeren, 2010, Frank and Loeffler, 2012, Weese, 2012). Some basic infection control guidelines for small animal veterinary medicine are readily available, e.g. those developed by the British Small Animal Veterinary Association (www.bsava.com) and the Federation of European Companion Animal Veterinary Associations (www.fecava.org).

1.1.2 Antimicrobial use and antimicrobial susceptibility testing

Misuse and overuse of antimicrobials are recognized as key drivers in antimicrobial resistance in human medicine and the consequences of antimicrobial use in veterinary practice cannot be expected to be different. Any use of antimicrobials, whether considered therapeutic or not, and prudent or otherwise, exposes bacterial pathogens and the commensal microbiota to varying concentrations of antimicrobial drug for variable times. This creates a selection pressure that can result in emergence of resistance or, if a resistant subpopulation is present, an increase in the abundance of resistant bacteria (WHO, 2014b, Guardabassi *et al*., 2004b, Weese *et al*., 2015, Johnson, 2002, Ogeer-Gyles *et al*., 2006b, Guardabassi and Prescott, 2015, Prescott *et al*., 2002).

The development of new antimicrobial drugs has over the past decades been slow, and bacteria are capable to develop a wide range of resistance mechanisms. The need to use the relatively few antimicrobials available wisely, and to slow down the current rate of antimicrobial resistance development and spread has been increasingly recognized in small animal and human medicine (Gould, 2009, Guardabassi and Prescott, 2015, Weese, 2008a, Lloyd, 2010).

As antimicrobial resistance increases, treatment of bacterial infections according to past clinical experience *(i.e.* empirical therapy) is becoming more difficult. Unnecessary or inappropriate usage of antimicrobials can delay the diagnosis of non-infectious causes of clinical signs, delay the resolution of an infection, and select for growth of resistant bacterial populations, including the causative pathogen resistant to the antimicrobial used. Other negative aspects include unnecessary adverse effects of the drug treatment, and increased costs, including repeated veterinary visits or prolonged hospitalization (Gould, 2009,

Guardabassi and Prescott, 2015).When bacterial culture and antimicrobial susceptibility are used, the guiding of treatment towards rational and prudent antimicrobial use in individual patients lowers the amount of unnecessary or inappropriate use of antimicrobials in respective animal species (Bartges, 2004, Guardabassi and Prescott, 2015, WHO, 2014b). As the need for antimicrobial treatment may be urgent, empirical treatment is sometimes indicated while waiting for culture and susceptibility results. Prudent use of antimicrobials therefore includes considering likely pathogens and their susceptibility patterns when choosing empirical treatment

The accumulated results of previous susceptibility testing from specific populations are invaluable when selecting such empirical therapy and to monitor trends in antimicrobial resistance. However, published surveillance reports on antimicrobial resistance in dogs are currently relatively few, and much baseline data on antimicrobial susceptibility needed to inform clinical therapy decisions as well as guide in policy recommendations is lacking. An increased surveillance of antimicrobial resistance would also permit the early detection of resistant strains and support investigation of outbreaks. The need for increased surveillance of antimicrobial resistance in companion animals, has been recognized. Importantly, such surveillance should include not only cultures from patients that are "worst case scenarios", but a broad, representative population so that the actual levels of antimicrobial resistance are reflected (Guardabassi and Prescott, 2015, Weese, 2008a, Prescott *et al*., 2002, WHO, 2014a, Weese, 2008c).

1.1.3 Interpretation and use of antimicrobial susceptibility results

A number of *in-vitro* antimicrobial susceptibility testing methods are available, and suitable for veterinary use, for example E-test, agar dilution, broth microdilution and disc diffusion. Comparison of such methods are outside of the scope of this thesis. However, it is worth noting that use of internationally accepted procedures is important not only as an in-house best-practice, but also to facilitate comparison of data from different studies (Schwarz *et al*., 2010, Dehaumont, 2004) .

Two different types of interpretive criteria are available: epidemiological cut-off values (www.eucast.org) and clinical breakpoints. Epidemiological cut-off values separate isolates without phenotypically reduced susceptibility, *i.e.* wild-type isolates, from isolates with reduced susceptibility without reference to clinical efficacy are suitable for monitoring purposes. Clinical breakpoints on the other hand are intended to predict clinical efficiency of the tested

antimicrobial for treatment of the tested bacterial species in an individual patient. When recommendations for clinical breakpoints are developed for laboratory use, the results of clinical efficacy studies, dosing and route of administration of the antimicrobial agents, the drug's pharmacokinetic and pharmacodynamic parameters in the respective animal species are taken into account (CLSI, 2008). The laboratory using these clinical breakpoints usually present the results to the clinician as a bacterial isolate being susceptible, resistant, or intermediate to the respective antimicrobial (Bywater *et al*., 2006, Schwarz *et al*., 2010).

Internationally accepted breakpoints for specific disease conditions caused by a particular bacterial species in defined animal host species are not always available. Furthermore, some results will be borderline intermediate-susceptible, or intermediate- resistant, although only reported as either susceptible or resistant. (Schwarz *et al*., 2010, Bywater *et al*., 2006).

The clinician has to use the susceptibility results from the laboratory responsibly. The clinical assessment is vital. For example, a satisfactory response to an antimicrobial treatment does not indicate a need for change of antimicrobial agent used despite the subsequent culture and susceptibility results yielding a pathogen intermediately resistant, to that agent. The relevance and likelihood of the bacterial species cultured actually being the cause of the clinical infection has to be taken into account. Resampling the infected site can be performed to address a seemingly discordant (incongruous) culture result vs. clinical suspicion, including cases where contamination of the sample is suspected.

The term multiresistance has been used inconsistently in the literature. It has been suggested that the term multiresistance exclusively should refer to acquired resistance properties, *i.e.* constitutive (innate, primary) resistance should not be included (Schwarz *et al*., 2010, Hoekstra and Paulton, 2002, Guardabassi and Prescott, 2015). Furthermore, to label an isolate as multidrug resistant, acquired resistance to three or more classes of antimicrobial agents should be detected. The prevalence of MDR pathogens in a study might increase or decrease depending on what antimicrobials are included in the investigation. This could in turn influence the clinicians' perception of the need for use of broad-spectrum antimicrobials. Investigations on the prevalence and importance of MDR pathogens should preferably exclude agents to which the bacteria are intrinsically resistant, as well as antimicrobials not relevant for treatment of the bacterial infection in question from a clinical viewpoint (Schwarz *et al*., 2010, Hoekstra and Paulton, 2002, Guardabassi and Prescott, 2015). For example, resistance to aminopenicillins in *E.coli* isolates in UTI is

of interest, but penicillin is not indicated for treatment of skin and soft tissue infections caused by *E.coli*.

1.2 Urinary tract infections

Bacterial urinary tract infection (UTI) is a common clinical problem in dogs and among the most common reasons for antimicrobial therapy, with approximately 14% of all dogs having at least one episode of UTI during their lifetime (Ling, 1984, Thompson *et al*., 2011a). Female dogs are more commonly affected than males. The by far most common form is a simple uncomplicated lower UTI, which is a sporadic bacterial infection of the bladder. A UTI can also include the upper urinary tract (upper UTI), most commonly the renal pelvis, as well as multiple sites, including the ureter, bladder, urethra, prostate, or vagina (Ling, 1984, Cohn *et al*., 2003, Thompson *et al*., 2011a, Ball *et al*., 2008, Seguin *et al*., 2003, Ling *et al*., 2001).

1.2.1 Complicated and recurrent urinary tract infections

The term complicated UTI has been used to describe both upper and lower UTI that occur in the presence of an anatomic or functional abnormality or a comorbidity that predisposes the patient to persistent infection, recurrent infection, or treatment failure. Conversely, the term uncomplicated UTI is used to describe UTI in patients where no underlying structural, neurologic, or functional abnormalities exist (Weese *et al*., 2011b, Ling *et al*., 1980, Jessen *et al*., 2015).

Most UTI in dogs are uncomplicated and occur as single episodes, and in cases of persistent and recurrent UTI predisposing factors can usually be identified. Examples of such predisposing factors include abnormal micturition, anatomic defects of the urinary tract, abnormal urothelium, altered urine composition or impaired immunity (Thompson *et al*., 2011a, Ling, 1984, Weese *et al*., 2011a). Recurrent UTI can also occur in dogs where no predisposing factors have been diagnosed (Ling, 1984, Weese *et al*., 2011b, Thompson *et al*., 2011a). In one study 4.5% of dogs with uncomplicated UTI had recurrent UTI (Seguin *et al*., 2003). Another retrospective study found only 0.3% of diagnosed UTI in dogs to be either recurrent or persistent (Norris *et al*., 2000).

Clinically applicable definitions of reinfection, relapse and refractory infections are presented in for example the Antimicrobial Use Guidelines for Treatment of Urinary Tract Disease from the International Society for Companion Animal Infectious Diseases (Weese *et al*., 2011b). Reinfection is defined as recurrence of a UTI within 6 months of cessation of previous,

apparently successful treatment and isolation of a different microorganism. Relapse is defined as recurrence of a UTI within 6 months of cessation of previous, apparently successful treatment and isolation of an indistinguishable organism from the one that was present previously, which is presumably because of failure to completely eliminate the pathogen. Furthermore, relapses can generally be expected to occur earlier than reinfections (i.e., within weeks rather than months) and they are characterized by a period of apparent bladder sterility during treatment. Finally, refractory infection is defined as persistently positive culture results during treatment despite *in vitro* susceptibility to the antimicrobial, with no period of elimination of bacteriuria during or after treatment (Weese *et al*., 2011b).

Differentiation of persistent infections, relapses or re-infection in clinical cases of canine UTI is often difficult. Ideally, genotyping should be included to investigate whether the same strain is present, in addition to identification of the bacterial species and investigation of the antibiograms. In clinical practice such analyses are, with rare exceptions, not available. Furthermore, changes in susceptibility can occur in individual strains, and different strains can be genotypically indistinguishable (Ball *et al*., 2008, Weese *et al*., 2011b).

Culture and susceptibility testing should be repeated in persistent- or re-infections, regardless of whether this has already been performed on previous bouts of UTI. Bacterial resistance to the antimicrobial administered is a possible contributing factor, or the major cause of lack of clinical improvement in persistent, relapsing or reoccurring UTI. The possibility of poor owner- or patient compliance should as always be included in the investigation of bacterial infections not responding as expected to the prescribed treatment. Notably, it has been shown in humans that the proportion of resistant *E coli* isolated from complicated urinary tract infections is significantly higher than the proportion isolated from uncomplicated cases. Furthermore, previous antimicrobial treatment of UTI could potentially have an impact on the resistance profiles of the dog´s resident bacterial flora, facilitating growth of resistant bacteria. The possibility of underlying abnormalities as well as of the bacteria being capable of evading host immune defence mechanisms should not be overlooked. The predisposing causes that complicate the UTI need to be diagnosed, managed and if possible eliminated (Thompson *et al*., 2011a, Weese *et al*., 2011a). The distinction between complicated UTI and inefficiently treated UTI determines the prognosis as well as recommendations for further and future investigations and treatments (Thompson *et al*., 2011a, Weese *et al*., 2011b).

1.2.2 Bacterial pathogens

Most UTI are thought to result from ascending infections, with the causative bacterial pathogens most often originating either from the gastrointestinal tract or from the skin surrounding the vulva and prepuce. The bacteria ascend via the urethra to the urinary bladder where they adhere and colonize the urothelial surface. Ascension of bacteria from the lower urinary tract is the primary route for upper UTI. Upper UTI can also, though rarely, be of haematogenous/lymphatic origin, or due to direct extension from surrounding tissues (Thompson *et al*., 2011a, Seguin *et al*., 2003, Bartges, 2004, Ling *et al*., 1980). It is therefore not unexpected that *E. coli*, *Staphylococcus pseudintermedius (S. pseudintermedius)*, *S. aureus*, beta haemolytic *Streptococcus* spp., *Proteus spp.*, *Enterococcus* spp., and *Klebsiella* spp. account for 95% or more of all urinary isolates in dogs (Seguin *et al*., 2003, Bartges, 2004, Ling *et al*., 2001, Weese *et al*., 2011a, Ogeer-Gyles *et al*., 2006a). The by far most commonly cultured bacterial species isolated from canine urine -as well as from human and feline UTI- is *E. coli* which in various studies has accounted for more than half of all positive urine cultures (Seguin *et al*., 2003, Bartges, 2004, Ling *et al*., 2001, Ogeer-Gyles *et al*., 2006a, Weese *et al*., 2011b).

1.2.3 Diagnosis

Clinical signs of UTI include dysuria, pollakiuria, and/or increased urgency of urination. The clinical signs are not pathognomonic for infection. The likelihood of the presenting complaints being due to clinically significant UTI has to be evaluated by the clinician. Urinalysis is useful in differentiating an uncomplicated lower UTI from other disorders causing the clinical signs, as well as in further investigations of possible underlying causes of UTI, such as endocrine disorders. A dipstick analysis is of value, as it often detects hematuria and proteinuria in cases of UTI. Notably, the dipstick analyses for nitrite (bacteria) and leukocyte esterase are designed for use in people and are not reliable tests for canine and feline patients. A urine sediment examination should therefore in addition be performed to identify pyuria and bacteriuria. Presence of hematuria, proteinuria and evidence of inflammation together with appropriate findings at clinical examination and clinical signs increase the suspicion of a bacterial infection of the urinary tract. As none of the findings are pathognomonic, including a urine culture as a part in the investigation still remains the definitive diagnostic test of UTI (Bartges, 2004, Weese *et al*., 2011b, Smee *et al*., 2013, Ball *et al*., 2008, Seguin *et al*., 2003). Bacterial

culture and susceptibility testing can not only confirm the presence of infection, but also aid in selection of relevant antimicrobial therapy.

Notably, detection of bacteria in urine samples occurs not only in clinical cases of UTI, but also in asymptomatic, or subclinical bacteriuria which can be defined as the presence of bacteria in the urine as determined by positive bacterial culture in the absence of clinical and cytological evidence of UTI. Numerous reports record the prevalence of bacteriuria in dogs with known underlying clinical disease (*e.g.* diabetes mellitus, hyperadrenocorticism, and urolithiasis) or in dogs after medical intervention (*e.g.* urinary catheterisation, immunosuppression). (Thompson *et al*., 2011b, Torres *et al*., 2005, Bubenik *et al*., 2007, McGhie *et al.*, 2014, Weese *et al*., 2011b, Smee *et al.*, 2013). Whether antimicrobial treatment should be administered to dogs with subclinical bacteriuria remains a controversial topic. In some cases, the bacteria present in animals with an asymptomatic bacteriuria may actually provide protection against colonization of the urinary tract with more pathogenic strains of bacteria (Thompson *et al*., 2011a, Barsanti, 2006). The Antimicrobial Use Guidelines for Treatment of Urinary Tract Disease from the International Society for Companion Animal Infectious Diseases states that treatment may be considered if there is concern that there is a particularly high risk of ascending or systemic infection (e.g., immunocompromised patients, patients with underlying renal disease) or that the bladder may be a focus of extra urinary infection. Importantly, treatment should not be used as a replacement for proper diagnosis and management of the underlying cause (Weese *et al*., 2011a).

The relevance of the identified species in UTI in dogs has to be included in interpretation of the significance of the detection of bacteria in a urine sample. In addition, colony counts (the level of bacterial growth), can be used as an aid. Quantitative aerobic bacterial culture of urine provides an estimate of the number of bacteria present. It has been suggested that as urine normally is sterile, any level of bacterial growth from samples collected by cystocentesis may be significant, provided that the sample collection was performed properly. It has also been proposed that urine from a UTI in dogs typically contain $\geq 10^3$ colony forming units (CFU)/mL. For samples collected via catheter, bacterial counts $\geq 10^4$ CFU/mL in male and $\geq 10^5$ in female dogs could be considered significant and for free-catch samples $\geq 10^5$ CFU/mL (Bartges, 2004, Comer and Ling, 1981, Weese *et al*., 2011b).

Although infection with more than one species can occur, detection of several potential pathogens should be interpreted with caution, as the majority (72-91 %) of UTI in dogs are caused by a single bacterial species (Ball et al., 2008, Rowlands et al., 2011, Weese et al., 2011b, Ling et al., 2001).

1.2.4 Collection and submission of urinary samples for bacterial analyses

Bacteria detected in a urine sample can also be present as a result of contamination of the sample. Poor sampling technique can lead to contamination with resident bacterial flora in the urethra, prepuce and vagina, a flora which includes potential pathogens of the sample. In veterinary patients, collection of urine for culture by cystocentesis is considered the gold standard as it avoids bacterial contamination with faecal bacteria and bacteria from the distal urogenital tract. However, it is not uncommon for both cystocentesis and second-best options in trying to avoid bacterial contamination of the sample, like catheterization or securing a midstream sample, to be unrealistic alternatives when collecting the sample due to either cost or practical problems (Ling *et al*., 1980, Rowlands *et al*., 2011, Comer and Ling, 1981, Thompson *et al*., 2011a).

Handling and processing of the collected sample can affect the culture results. Time from sample occasion to processing may in itself affect the culture results, including assessment of yielded growth on the agar plates. Immediate culture (within four hours) after sampling is ideal to avoid false-positive and false- negative culture results. Both rapid multiplication of bacteria (doubling in number in about 45 minutes) and a decrease in number has been reported from studies investigating urine samples kept at room temperature (Ling *et al*., 1980, Lulich and Osborne, 2004, Tivapasi *et al*., 2009). In-house culture should however not be attempted if the knowledge and practical settings for culture and identification of bacterial species and their antimicrobial susceptibility patterns are lacking. In a practice setting, bacterial samples are therefore often referred to an external laboratory for culture and susceptibility. The optimal submission method minimises misleading results, by restricting growth of contaminants while preserving pathogens (Ling *et al*., 1980, Tivapasi *et al*., 2009, Smee *et al*., 2013, Weese and Jalali, 2014).

Recommendations for storing urine samples that are not immediately cultured include refrigeration within 1 or 2 hours of collection. Refrigeration (4°C) of the samples might be beneficial not only during temporary storage but also during shipping. One study recorded that quantitative bacterial counts differed after 6 hours of refrigerated storage, but without a change in interpretation of the clinical significance, and concluded that it is acceptable for urine to be refrigerated in a closed container for up to six hours prior to culture (Padilla *et al*., 1981). On the other hand, false negative results (failure of bacteria to grow) might also occur when specimens are refrigerated for long periods of time. In clinical practice, samples are usually not refrigerated during

transportation (Penna *et al*., 2010, Rowlands *et al*., 2011, Weese *et al*., 2011b, Tivapasi *et al*., 2009).

Submission of the sample as preincubated media; agar plates or urine dipstick paddles for veterinary use with an appropriate medium is another possible way of supporting maintenance of relevant microorganisms without overgrowth. Dipstick paddles consist of a culture paddle embedded with 2 standard culture media. Preincubation at the clinic gives the clinician the possibility to screen samples for bacterial growth, as part of the investigation of UTI, and subsequently submit only positive culture for further analyses. After 24 hours incubation the dipstick paddles/agar plates with positive growth can be sent to an appropriate microbiology laboratory (Ybarra *et al*., 2014, Weese *et al*., 2011b, Smee *et al*., 2013, Ling *et al*., 1980, Ling *et al*., 2001).

In human medicine, a relatively high overall sensitivity and specificity for detection of relevant bacteriuria has been reported for urinary dipslides (e.g. approximately 70% and 82-94% respectively) when compared to cultures performed in a diagnostic microbiology laboratory (Anacleto *et al*., 2009, Winkens *et al*., 2003, Scarparo *et al*., 2002). A recent study of a commercially available veterinary dipslide system showed similar results (Ybarra *et al*., 2014).

Relevant publications on the use of bacterial swabs specifically for canine urinary samples are lacking. A few studies have investigated the use of boric acid-glycerol-sodium formate tubes for submission of urine samples for culture, and compared the culture results to immediate culture of fresh urine, as well as either culture from urine kept in a sterile plastic tube or in a dipslide tube, with conflicting results (Rowlands *et al*., 2011, Perrin and Nicolet, 1992). Rowlands and co-workers concluded that urine samples should be submitted to the laboratory in a plain sterile tube (Rowlands *et al*., 2011). In the study by Perrin and co-workers, preserving samples in boric acid was beneficial. Samples sent in the boric acid and in the dip-slide tube showed comparable culture results, while culture of the samples sent in a sterile plastic tube yielded 53% false positive results in comparison with those of the samples preserved in boric acid (Perrin and Nicolet, 1992).

Ling and co-workers reported that *Proteus* spp. were isolated more frequently from urine specimens collected by catheterisation or midstream catch than by cystocentesis, and in a study by Bubenik and co-workers, *Enterobacter* spp. and *Staphylococcus* spp. were more frequently isolated from catheterised dogs (Bubenik *et al*., 2007, Ling *et al*., 2001). To what extent the prevalence of various bacterial agents is influenced by sample technique and sample material is otherwise not well described, and further studies specifically designed to compare sample methods and sample materials are warranted.

Other factors that have been reported to possibly influence the distribution of bacterial pathogens in urine cultures include the dogs' gender, urine concentration and of acidity of the urine, but the results published are conflicting (Norris *et al*., 2000, Cohn *et al*., 2003, Tivapasi *et al*., 2009, Ling *et al*., 2001).

1.3 Surgical site infections

Infection is an inherent risk of surgery, and surgical site infections (SSI) cannot be expected to be completely eliminated. Some SSI are preventable, others are not, for example due to the surgical area being infected or contaminated prior to an emergency surgery (Nelson, 2011, Mangram *et al*., 1999, Eugster *et al*., 2004).

Surgical site infections are among the most common nosocomial infections in human patient populations (Barnett, 2007, Mangram *et al*., 1999). They account for 16% of nosocomial infections in all patients and 38% of nosocomial infections among surgical patients in the United States (Mangram *et al*., 1999). SSI has been described as a complication of 3.6% to 18.1% of small animal surgical procedures with significant variation associated with surgery type; infection rates for clean surgical procedures tend as expected to be in the lower range: from 3.6% to 5.8% (Eugster *et al*., 2004, Vasseur *et al*., 1988, Vasseur *et al*., 1985, Weese and Halling, 2006, Frey *et al*., 2010, Brown *et al*., 1997, Whittem *et al*., 1999, Weese, 2008c). SSI can affect the success of initial surgical intervention and delay healing. Furthermore they can incur additional costs (Turk *et al*., 2015, Nelson, 2011, Nicoll *et al*., 2014). Although development of SSI is multifactorial, and published scientific data on prevention of SSI in small animal medicine relatively limited, much information on key factors in preventing SSI can be extrapolated from human medicine (Nelson, 2011, Weese, 2008c, Mangram *et al*., 1999).

As for other HAI, development and implementation of proper infection control programs to prevent spread of antimicrobial resistant bacteria in clinics and hospitals, as well as surveillance protocols including patterns of antimicrobial resistance have in human medicine been shown to be key components in reducing SSI rates. Well-known key preventive measures specifically aimed at reducing the risk of SSI include adherence to aseptic principles and good surgical techniques as well as proper preparing and caring of the dog and the surgical area prior to, during, and after surgery (Nelson, 2011, Dohmen, 2008, Mangram *et al*., 1999, Beal *et al*., 2000, Brown *et al*., 1997, Barnett, 2007, Eugster *et al*., 2004).

Surgical site infections are, as other bacterial HAI, increasingly complicated by the emergence of MDR bacteria. The risk of SSI developing due to contamination with resident environmental MDR bacteria in health care facilities is well known in human medicine. In small animal medicine the risk of MDR bacteria causing SSI in dogs has been increasingly recognized. Although the morbidity and mortality associated with MDR SSI have not been thoroughly investigated in small animal practice, it is reasonable to assume that they, as shown in human medicine, are associated with poorer outcomes including increased mortality (Wieler *et al*., 2011b, Weese, 2008c, Dohmen, 2008, Barnett, 2007, Owens and Stoessel, 2008, Bergstrom *et al*., 2012, Weese *et al*., 2012, Nicholson *et al*., 2002, Bratzler *et al*., 2013).

1.3.1 Bacterial pathogens

In humans, the patients' endogenous flora is the major source of bacteria infecting surgical wounds (Mangram *et al*., 1999). The endogenous skin flora is also recognized as an important cause of SSI in dogs, as aseptic preparation of the skin cannot completely eliminate skin-associated bacteria, especially not bacteria residing in the deeper parts of the skin such as the hair follicles and sebaceous glands. Both superficial and deeper infection can be the result, as the bacteria can enter deeper tissues during the initial incision. Bacterial species known to be part of the canine skins endogenous flora, as well as pathogens important in canine dermatitis are therefore expected to be prevalent findings in canine SSI (Nelson, 2011, Johnson, 2002, Mangram *et al*., 1999). These include staphylococci, of which *S. pseudintermedius* is the by far most frequent bacterial pathogen in canine dermatitis- one of the most common reason for antimicrobial treatment in dogs, and for dog owners seeking veterinary care for their animal. Besides *S. pseudintermedius*, the most clinically relevant staphylococci in canine medicine are *S. aureus*, and the species more recently recognized as a pathogenic species implicated in canine infections *Staphylococcus schleiferi subsp. coagulans* (*S. schleiferi*) (Bannoehr and Guardabassi, 2012, Devriese *et al*., 2005, Frank *et al*., 2003, May *et al*., 2005, Morris *et al*., 2006, Cox *et al*., 1984)

Commensal flora expected to be present if the surgical area or procedure involves gut, genital and urinary tract, or respiratory tract include *E.coli*, *Proteus spp.*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp. and beta haemolytic *Streptococcus* spp. (Nelson, 2011, Weese, 2013, Priestnall and Erles, 2011, Quinn P.J., 2011). Although these bacterial species are less commonly associated with pyoderma than staphylococi, they are cultured from healthy and diseased canine skin and are recognized as pathogens in skin

disease. Endogenous flora can also be transferred to intact skin at the planned incision site before the procedure, for example through the dogs grooming behaviour (Nelson, 2011, Weese, 2013, May, 2006, Priestnall and Erles, 2011, Turk *et al*., 2015, Nicoll *et al*., 2014, Summers *et al*., 2012, Rodrigues Hoffmann *et al*., 2014, Hillier *et al*., 2014, Quinn P.J., 2011, Eugster *et al*., 2004).

Exogenous sources of surgical contamination include the surgical equipment, the physical environment and bacteria on hands and clothes of personnel. In addition, bacterial contamination of affected tissues can also stem from for example a traumatic injury, including bite wounds (Nelson, 2011, Johnson, 2002).

The frequency of exogenous versus endogenous flora causing SSI in dogs is not well described. Many of the risk factors that might contribute to exogenous infection such as poor surgical technique (skill of the individual surgeon), lack of aseptic preparation of the surgical team, inappropriate ventilation in the operation theatre and other environmental factors described in human medicine are probably applicable to veterinary medicine. Some risk factors, such as numbers of people in the operating room, student surgeon, prolonged anaesthetic and surgical time, duration of hospitalization and drain placement have also been described as associated with SSI in dogs, but knowledge of the relative importance of each of these factors is relatively limited (Boerlin *et al*., 2001, Nelson, 2011, Weese, 2008c, Vasseur *et al*., 1985, Vasseur *et al*., 1988, Beal *et al*., 2000, Brown *et al*., 1997, Nicholson *et al*., 2002, Heldmann *et al*., 1999, Mangram *et al*., 1999).

1.3.2 Diagnosis

The diagnosis of SSI requires interpretation of both clinical and laboratory information and is subject to a certain degree of subjectivity. The clinical distinction between infection and inflammation can be difficult, as some local inflammation or serous discharge can be expected at the incision site of a surgical procedure. The clinical signs due to inflammation may be indistinguishable to those of an added infection. Various definitions have also been used for postoperative SSI in veterinary medicine publications (Eugster *et al*., 2004, Nelson, 2011, Vasseur *et al*., 1988, Whittem *et al*., 1999, Billings *et al*., 1990, Brown *et al*., 1997). Bacterial culture is important to verify an ongoing infection, as well as, together with antimicrobial susceptibility testing, guide in selecting an appropriate antimicrobial therapy, if such therapy is necessary.

Surgical wound criteria intended to aid in comparisons between studies and for surveillance of SSI have been developed by among others The Centers for Disease Control and Prevention National Nosocomial Infections Surveillance (NNIS) (Mangram *et al*., 1999) and The US Centers for Disease Control and Prevention (CDC) (Horan *et al*., 1992). A clear correlation between SSI rates and four described categories of wound contamination; clean, clean-contaminated, contaminated, and dirty has been reported in human medicine (Mangram *et al*., 1999, Horan *et al*., 1992). The CDC and NNIS classify SSI as superficial incisional, deep incisional or organ/space (Horan *et al.*, 1992, Mangram et al., 1999). When evaluating SSI in dogs an objective definition is desirable for facilitating comparison of veterinary studies. However, such classifications have rarely been used in publications on canine SSI (Weese, 2008c, Eugster e*t al*., 2004, Nelson, 2011).

Empirical antimicrobial treatment might be indicated while awaiting culture- and susceptibility results (Nelson, 2011, Nicholson *et al*., 2002). Furthermore, perioperative antimicrobial treatment is widely used to prevent SSI. The goal of perioperative antimicrobial therapy is to reduce the risk of infection while having minimal negative impact on the patient's microflora and minimizing the risk of antimicrobial-associated complications. Standard guidelines in human medicine state that antimicrobials should be administered intravenously so that there are adequate serum and tissue concentrations at the time of surgery and at a most a few hours after the procedure (Mangram *et al*., 1999, Bratzler *et al*., 2013, Bratzler *et al*., 2005). Less information is available in veterinary medicine. Concerns with postoperative antimicrobial administration are possible limited benefits in the prevention of SSI paired with an increased risk of the development of MDR infections. As conflicting results regarding the benefit of perioperative treatment in various types of surgical procedures are present, the use of antimicrobials remains controversial *e.g.* in clean procedures (Nelson, 2011, Weese, 2008c, Eugster *et al*., 2004, Bratzler *et al*., 2013, Weese and Halling, 2006, Brown *et al*., 1997, Vasseur *et al*., 1985, Vasseur *et al*., 1988).

As mentioned above, empirical treatment is becoming more difficult as antimicrobial resistance increases, and the increased awareness of the risk of nosocomial infections with MDR bacteria might pressure attending veterinarians to use antimicrobials other than traditional first-line antimicrobials (Guardabassi *et al*., 2004b, Weese, 2008a, Guardabassi and Prescott, 2015, Wieler *et al*., 2011b). However, the focus in veterinary studies on SSI in dogs have tended to be directed towards investigating possible risk factors for developing an infection, and few recent veterinary studies have investigated the relative growth of pathogenic bacteria, including susceptibility

patterns of the isolated pathogens in canine SSI where less complicated procedures are included (Eugster *et al*., 2004, Nicholson *et al*., 2002, Beal *et al*., 2000, Billings *et al*., 1990, Brown *et al*., 1997, Frey *et al*., 2010, Heldmann *et al*., 1999, Mayhew *et al*., 2012, Vasseur *et al*., 1985, Vasseur *et al*., 1988, Weese and Halling, 2006, Whittem et al., 1999, Turk *et al*., 2015, Nicoll *et al*., 2014).

1.4 Methicillin-resistant *Staphylococcus pseudintermedius*

Staphylococcus intermedius was first described in 1976 (Hajek, 1976). More recent work showed that isolates phenotypically identified as *S. intermedius* were three distinct species, *S. intermedius, S. pseudintermedius* and *Staphylococcus delphini*. These three species are together referred to as the *Staphylococcus intermedius* group (SIG) (Bannoehr *et al*., 2007, Devriese *et al*., 2005, Sasaki *et al*., 2007b). *Staphylococcus pseudintermedius* is a skin and mucous membrane commensal as well as the most important staphylococcal pathogen in dogs. It has also been labelled the overall most frequently bacterial pathogen isolated from clinical canine specimens. As mentioned above it is the by far most frequent bacterial pathogen in skin- and ear infections (Bannoehr and Guardabassi, 2012, Devriese *et al*., 2005, May *et al*., 2005), and canine dermatitis is one of the most common reasons for antimicrobial treatment in dogs, as well as for dog owners seeking veterinary care for their animal. *Staphylococcus pseudintermedius* is also recognized as an important pathogen in various soft tissue infections (Bannoehr and Guardabassi, 2012, Frank *et al*., 2003, May *et al*., 2005, Morris *et al*., 2006, Cox *et al*., 1984).

Staphylococcus pseudintermedius is traditionally identified by colony morphology and standard phenotypic tests. Owing to a lack of unique biochemical markers, differentiation between the members of the SIG is difficult unless genotypic methods are used (van Duijkeren *et al*., 2011a, Bannoehr and Guardabassi, 2012). Several methods are available, and used in various publications including a multiplex PCR method for species identification of coagulase-positive staphylococci targeting the *nuc* gene locus, matrix assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) (Bannoehr and Guardabassi, 2012, van Duijkeren *et al*., 2011a). However, as the vast majority of canine strains are S. *pseudintermedius*, the risk of species misidentification in routine diagnostics is low for canine specimens. Furthermore, the impact that such sporadic misidentification may have on clinical practice and patient care are probably

negligible. After the reclassification of *S. intermedius* to *S. pseudintermedius*, it has therefore been proposed that all canine isolates should be termed *S. pseudintermedius*, unless proven otherwise by genetic typing methods (Devriese *et al*., 2009, Bannoehr and Guardabassi, 2012).

Methicillin-resistant *S. pseudintermedius* (MRSP) was first reported in 1999, from North America, and the first report on methicillin-resistant *S. pseudintermedius* (MRSP) in Europe was published in 2007 (Loeffler *et al*., 2007, Gortel *et al*., 1999). Methicillin resistance in *S. pseudintermedius*, as well as in *S. aureus* is mediated by the *mec*A gene, which encodes a penicillin-binding protein (PBP2a) that has a low affinity for all ß-lactam antimicrobials (Chambers, 1997, Kwon *et al*., 2006). This means that MRSP and MRSA strains are resistant to all beta-lactam antimicrobials, *i.e.* penam penicillins, including clavulanic acid potentiated preparations, cephalosporins and carbapenems which are considered to be last resort antimicrobials in human medicine (Frank and Loeffler, 2012, WHO, 2012). The *mec*A gene has also been identified in other staphylococci, including *S. schleiferi* (Loeffler *et al*., 2007, Cain *et al*., 2011).

Most publications on MRSP have focused on isolates from dogs, and only few isolates from other animals have been investigated. MRSP has however been isolated from cats, and some other animal species including horses. Cats are less frequently colonized by *S. pseudintermedius*, less often suffering from staphylococcal infections, and are therefore expected to also be less frequently colonized and infected with MRSP (Wettstein *et al*., 2008, Morris *et al*., 2006, Ruscher *et al*., 2009, Kadlec and Schwarz, 2012, Nienhoff *et al*., 2011).

Resistance to oxacillin or cefoxitime is used as an indicator of methicillin resistance, but the final identification of MRSP and MRSA isolates is based on the presence of the *mec*A gene. Detection of *mec*A via polymerase chain reaction (PCR) has been recommended for diagnosing methicillin resistance (van Duijkeren *et al*., 2011a, Kadlec and Schwarz, 2012).

Isolation of MRSP from dogs has been reported with increasing frequency worldwide (Weese and van Duijkeren, 2010, Lehner *et al*., 2014, van Duijkeren *et al*., 2011a, Ruscher *et al*., 2010, Moodley *et al*., 2014). Reported prevalence rates of MRSP positive dogs in the community, or specifically in dogs with skin disease vary widely, e.g. 0-7% in dogs (Griffeth *et al*., 2008, Vengust *et al*., 2006, Kania *et al*., 2004, Hanselman *et al*., 2008, Hanselman *et al*., 2009). In one study from a veterinary clinic in Japan, 30% of sampled dogs were MRSP positive (Sasaki *et al*., 2007a) (Sasaki *et al*., 2007a), and in two studies on dogs with pyoderma in China and Japan MRSP prevalence was 48% and 66% respectively (Feng *et al*., 2012, Kawakami *et al*., 2010).

In Sweden, data on resistance in *S. pseudintermedius* in isolates from Swedish dogs referred to the National Veterinary Institute from veterinary clinics and hospitals has been reported since 1992, and since 2008, methicillin resistant coagulase positive staphylococci are notifiable in Sweden. The first MRSP isolates in Sweden were described in the year 2006, when 13 dogs were confirmed to be infected with MRSP. The number of confirmed canine MRSP infections quickly increased. In 2009, more than 120 MRSP isolates were confirmed and hence reported (SVARM, 2009). However, after the peak in number of isolates in 2009, the number of isolates detected per year decreased, and during the year 2013 in total 33 isolates were reported (SWEDRES-SVARM, 2013, SVARM, 2011).

DNA-based investigations of MRSP isolates including use of multi-locus sequencing (MLST), have shown that MRSP isolates belonged to five distinct cloneal lineages, labelled sequenced types (ST) numbers ST29, ST68, ST69, ST70 and ST71, and that MRSP clones were not shared between Europe and North America. Furthermore, within the five ST- types two predominant lineages have been identified; ST68 in the USA and ST71 in Northern Europe (Bannoehr *et al*., 2007, Perreten *et al*., 2010, Ruscher *et al*., 2010). In contrast, methicillin-susceptible *S. pseudintermedius* (MSSP) is, based on current knowledge, not regarded as being clonally distributed, and it has been postulated that the *mec*A gene has been acquired by different *S. pseudintermedius* strains (Bannoehr *et al*., 2007, Bannoehr and Guardabassi, 2012, Black *et al*., 2009). It is also thought that the *mec*A gene is likely to have originated from coagulase negative staphylococci (members of the *Staphylococcus sciuri* group) that colonize both animals and humans (Tsubakishita *et al*., 2010, Frank and Loeffler, 2012).

It is considered unlikely that the rapid emergence and spread of MRSP is due to frequent transmissions of *SCCmec*, the genetic element that contains *mec*A. Differences in the genetic makeup of antimicrobial resistance in MRSP and MSSP, including difference in resistance genes conferring resistance to tetracyclines and macrolides, together with the dominance of a few MRSP clones points towards a successful spread of MRSP clones, as opposed to frequent acquisition of methicillin resistance in MSSP. MRSP and MRSA are thought to have evolved separately through adaptation to their respective hosts. MRSP emerged several decades after emergence of MRSA in human medicine and some of the *SCCmec* types found in MRSP have never been described in *S. aureus* (Bannoehr and Guardabassi, 2012, Guardabassi *et al*., 2004b, Perreten *et al*., 2010, Bannoehr *et al*., 2007).

1.4.1 Clinical implications of canine MRSP infection

Investigations of clinical presentations of MRSP infection are scarce, but there is currently no evidence that MRSP is more invasive or able to cause different types of disease than MSSP. Published clinical reports on MRSP infections have mirrored infections known to be caused by MSSP. The majority of reported MRSP infections have been superficial and deep pyoderma, ear and wound infections, and postoperative infections. Other reported infections include urinary tract infections and respiratory infections (Beck *et al*., 2012, van Duijkeren *et al*., 2011a, Jones *et al*., 2007, Frank and Loeffler, 2012, Weese and van Duijkeren, 2010).

Case analyses have to date not indicated any differences in severity and outcome between MRSP and MSSP infections, but studies of virulence factors in MRSP and MSSP are scarce (Loeffler *et al*., 2007, Morris *et al*., 2006, Weese *et al.*, 2012, Fitzgerald, 2009, Bryan *et al*., 2012, Bannoehr and Guardabassi, 2012).

Despite the lack of indications of differences in severity and outcome between canine MRSP and MSSP infections, MRSP infections present significant clinical challenges to veterinary surgeons. In addition to the broad range of important antimicrobials that are beta-lactam antimicrobials, MRSP strains often, in addition to *mec*A, carry a wide range of other antimicrobial resistance genes that confer resistance to almost all classes of antimicrobial agents routinely used in small animal practice. Often no clinically relevant pet authorized systemic antimicrobial drugs are effective (Kadlec *et al*., 2010, Perreten *et al*., 2010, Weese and van Duijkeren, 2010, van Duijkeren *et al*., 2011a).

The limited choice of effective veterinary antimicrobial agents available for treatment of MRSP is well recognized in Sweden, where more than 90% of MRSP isolates investigated between the year 2009 and 2011 were susceptible only to two of the antimicrobials licensed for use in dogs; fusidic acid and tetracycline. Some isolates have been resistant to tetracycline as well (SVARM, 2011).

The few antimicrobials left for treatment of MRSP infections include antimicrobials such as linezolid, quinupristin/dalfopristin, rifampicin, vancomycin and local treatment with mupirocin; antimicrobials that are used for decolonization or as last resort antimicrobials against methicillin-resistant staphylococci in humans. Use of such antimicrobials in dogs is therefore controversial (WHO, 2012, Weese, 2008b, Guardabassi *et al*., 2004b, Weese *et al*., 2015, Frank and Loeffler, 2012). The national guidelines on clinical use of antibiotics in dogs and cats from the Swedish Veterinary Association (www.svf.se) stated in the year 2009 that such antimicrobials should only be

used if necessary for animal welfare and where no other treatments are available. Furthermore, a specialist competence is to be involved, and the motivation for such treatment should be noted in the medical chart. The use of antimicrobials has subsequently been further restricted through legislation (The Swedish Board of Agriculture, www.jordbruksverket.se)

Other antimicrobials include chloramphenicol, which is rarely used due to possible side effects, and to which European MRSP isolates often are resistant. European MRSP isolates are rarely susceptible to clindamycin, or fluoroquinolones (Kadlec and Schwarz, 2012, Perreten *et al*., 2010, Weese and van Duijkeren, 2010, van Duijkeren *et al*., 2011a, Kadlec *et al*., 2010, Guardabassi *et al*., 2004b, Guardabassi and Prescott, 2015). Furthermore, use of fluoroquinolones has been associated with an increased risk for isolation of MRSA. The use in treatment of MRSP is therefore controversial (Frank and Loeffler, 2012, Guardabassi and Prescott, 2015).

Topical therapy include using shampoos containing chlorhexidine, benzoyl peroxide or ethyl lactate, and treatment of focal lesions with chlorhexidine spray, benzoyl peroxide gel, fusidic acid or nisin. As with any antimicrobial, prudent use of topical products is indicated to prevent widespread resistance (Frank and Loeffler, 2012, van Duijkeren *et al*., 2011a).

1.4.2 Zoonotic aspects

Colonization with MSSP is uncommon in humans, even among people with frequent contact with animals. The low prevalence could be due to humans not being natural hosts for *S. pseudintermedius* as staphylococcal colonization in humans is dominated by *S. aureus* and *Staphylococcus epidermidis* (van Duijkeren *et al*., 2011a, Weese and van Duijkeren, 2010, Frank and Loeffler, 2012, Hanselman *et al*., 2009). Rare cases of human infections with MSSP are reported, usually associated with dog-bite wounds (van Duijkeren *et al*., 2011a, Weese and van Duijkeren, 2010, Talan *et al*., 1989, Sasaki *et al*., 2007a). MRSP has been cultured from persons living in households together with a dog with an ongoing or previous MRSP infection, as well as in personnel working in veterinary clinics where MRSP infected pets were treated infrequently. The prevalence of MRSP in humans living in households with dogs or working at small animal clinics has in various studies varied from less than one percent to 14 % (Morris *et al*., 2010, Paul *et al*., 2011, Frank *et al*., 2009, Laarhoven *et al*., 2011, Ishihara *et al*., 2010). The presence of genetically similar, or indistinguishable isolates in pets and their owners has in some studies been confirmed (Guardabassi *et al*., 2004a, Soedarmanto *et al*., 2011, Zubeir *et al*., 2007, van Duijkeren *et al*., 2008). As colonization of humans is thought to be

infrequent and transient, the risk of zoonotic transmission causing clinical infection with MRSP in humans is considered to be small. As for MSSP, reports on MRSP infections in humans are also rare. Still, persons colonized with MRSP may have a higher risk of developing MRSP infections in case of surgical or non-surgical wounds. The real incidence of MSSP and MRSP infections in humans may also be underestimated due to lack of awareness in human laboratories, as they can be misidentified as methicillin susceptible *S. aureus* (MSSA) or MRSA in routine diagnostics (van Duijkeren *et al*., 2011a, Starlander, 2014, Pottumarthy *et al*., 2004, Talan *et al*., 1989).

1.4.3 Detection and differentiation of MRSP contamination and colonization

MRSP positivity can be the result of a true MRSP colonization or of contamination, as well as of an MRSP infection. The term carrier is used to describe an individual colonized with MRSP. Colonization is the presence, growth and multiplication of MRSP in one or more body sites without observable clinical signs or immune reaction. The word contamination is used as when the word is used in everyday life; bacteria contaminating a dog can easily be washed off. The term infection is used to describe a condition where MRSP has invaded a body site, is multiplying in body tissue, and is causing clinical manifestations of disease (van Duijkeren *et al*., 2011a). Both animals and humans can be contaminated, colonized or infected with MRSP (van Duijkeren *et al*., 2011a).

Only a few longitudinal studies of MRSP involving repeated cultures of the same individuals have been published (Bergstrom *et al*., 2012, Laarhoven *et al*., 2011, Windahl *et al*., 2012). Most studies on MRSP are one-point studies with only one sample per individual, and it is unclear if the investigated dogs were colonized persistently or if they were contaminated with MRSP. Furthermore, studies investigating carriage of MSSP are also scarce (van Duijkeren *et al*., 2011a, Bannoehr and Guardabassi, 2012). In a review by Bannoehr and co-workers (Bannoehr and Guardabassi, 2012), the authors found only three longitudinal studies on MSSP carriage in healthy adult dogs. In two of these, carriage was found to be persistent (Saijonmaa-Koulumies and Lloyd, 2002, Cox *et al*., 1988). Hartman and co-workers found persistent carriage to be associated with higher numbers of MSSP at the colonization site (Hartmann *et al*., 2005). Bannoehr and co-workers concluded that longitudinal studies using appropriate sampling schemes and up-to-date methods for identification of MSSP are needed to classify carriage patterns as persistent carriage, intermittent carriage and non-carriage (Bannoehr and Guardabassi, 2012).

As MRSP positivity can be either the result of a true MRSP colonization or of contamination and as knowledge of long-term carriage of MRSP to date is limited, it is difficult to certifiably discriminate between the two. Even when MRSP is found in repeated cultures over time from the same dog, continuous carriage as opposed to intermittent carriage with reinfection is difficult to prove in field studies. In fact, in the majority of studies on MRSP in dogs, it is unclear if dogs are colonized persistently or if they were only transiently carrying the bacterium (van Duijkeren *et al*., 2011a, van Duijkeren *et al*., 2011b, Laarhoven *et al*., 2011). It has been suggested that as perineum is thought to be a primary colonization site for MSSP, MRSP positive cultures from perineal samples could be interpreted as colonization of the sampled dog being likely, but studies investigating this further are so far lacking (Devriese and De Pelsmaecker, 1987, van Duijkeren *et al*., 2008, van Duijkeren *et al*., 2011a).

Various DNA-based techniques have been used in strain typing of MRSP in surveillance and investigations of outbreaks. Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory methods for bacterial typing of genetic relatedness and has generally been the preferred method, or included among other methods, when comparing MRSP, and MSSP, isolates cultured within dog families, households and veterinary clinics, including samples from persons and environmental samples. Similar or indistinguishable PFGE patterns are interpreted as persistent carriage, or transmission of the same clone between dogs, persons and the environment in respective household or clinic (Guardabassi *et al*., 2004a, Soedarmanto *et al*., 2011, Zubeir *et al*., 2007, van Duijkeren *et al*., 2008, Paul *et al*., 2012, Loeffler *et al*., 2007, van Duijkeren *et al*., 2011a, Bannoehr and Guardabassi, 2012). Still, PFGE may lack the resolution needed to discriminate within a single clone in an outbreak or transmission within a household (Goering, 2010). For example, the clone ST71-t02-SCCmedII-III has shown a similarity of 80% or more on both a national and European level when compared using PFGE (Perreten *et al*., 2010, Ruscher *et al*., 2010, Borjesson *et al*., 2012).

As most studies on MRSP are one-point prevalence studies, to what extent genetic changes occur over time within a carrier is unclear. Notably, a high variability of PFGE patterns among MSSP isolates from both healthy and infected dogs has been reported (Fazakerley *et al*., 2010). Genotypic shifts have also been observed in MSSP isolates from persistently colonized dogs, and differences in antimicrobial susceptibility phenotypes may exist among strains isolated from different anatomical sites of the same dog (Hartmann *et al*., 2005, Bannoehr and Guardabassi, 2012).

Studies involving environmental sampling of households with MRSP positive animals have shown widespread contamination of the environment (van Duijkeren *et al*., 2011b, Laarhoven *et al*., 2011). The majority of MRSP positive environmental samples in these studies were from areas with physical contact with the index case, such as the feeding and sleeping place, indicating that physical contact is an efficient way of MRSP transmission. The bacteria were however also found in sites where there was little or no physical contact with the index case or contact pets (e.g. the floor underneath the sofa), indicating that dust particles (e.g. hairs, epithelial cells) carry MRSP to those sites. Duijkeren and co-workers found that it was more common for environmental samples to be positive in households in which the index case was MRSP positive than if the index dog was negative. Households where the index case still had a clinical MRSP infection had even more positive environmental samples (van Duijkeren *et al*., 2008, Laarhoven *et al*., 2011).

There are currently no guidelines on when, or if, a dog can be declared a non-carrier of MRSP. The risk of MRSP carriage being present, but not detected through sampling is unknown. Reference- or "gold" standards for screening of MRSP in animals are lacking both regarding laboratory procedures and what sample sites should be used (Beck *et al*., 2012, van Duijkeren *et al*., 2011a).

Sampling of multiple body sites, and relevant sites, *i.e.* sites where MRSP is most likely found, is key in increasing detection sensitivity (Laarhoven *et al*., 2011, Paul *et al*., 2012, Rubin and Chirino-Trejo, 2011). A minimum of both a nasal and rectal or perineal swabs has been recommended when screening for MRSP or MSSP (Weese and van Duijkeren, 2010, Rubin and Chirino-Trejo, 2011).

Published data on comparison of relative prevalence of positive MRSP cultures between various sample sites in dogs screened for MRSP carriage are scarce. Various combinations of sample sites have been used, including nose and perineum, or nose, perineum and infected sites, or nose, perineum and skin, with or without pooling of samples. The most commonly reported site of MRSP colonization in dogs is the nose and the anus, but these are also the most commonly tested sites (van Duijkeren *et al*., 2011a, Weese and van Duijkeren, 2010, Bergstrom *et al*., 2012, Hanselman *et al*., 2009, Nienhoff *et al*., 2011, Beck *et al*., 2012).

MSSP is known to colonize canine skin, hair follicles and coat, but in particular mucocutaneous sites, such as the nose, mouth and anus and the conjunctival sac. The nares, oral cavity and anal mucosa have been postulated to be the source of the staphylococcal population that colonizes the skin (Devriese and De Pelsmaecker, 1987, Beck *et al*., 2012). Bannoehr and co-

workers reviewed published data on MSSP carriage in dogs and approximated the reported carriage rates per body sites to be as follows (ranges in parenthesis: nose: 31% (16-64%), mouth: 57% (42-74%), groin: 23% (16-38%), perineum-rectum 52% (28-72%) (Bannoehr and Guardabassi, 2012). The authors concluded that, in contrast to *S. aureus* in humans, where nasal swabs are routinely used for screening purposes, canine carriers of S. *pseudintermedius* are most reliably be identified by swabbing both the oral mucosa and the perineum. The authors also pointed out that the hygiene and social behaviour patterns of dogs differ from humans, and frequent exposure and promiscuous exchange of this bacterium is likely in the dog population.

1.4.4 Risk factors for MRSP colonization and infection

Studies on the risk factors for MRSP colonization or infection are scarce, but medical treatment or antimicrobial therapy, skin lesions or wounds and symptoms of infection including dermatitis and veterinary visits or hospitalization have in various studies been associated with an increased risk of MRSP colonization or infection in dogs (Lehner *et al*., 2014, van Duijkeren *et al*., 2008, Bergstrom *et al*., 2012, Zubeir *et al*., 2007, Morris *et al*., 2010, Nienhoff *et al*., 2011, Sasaki *et al*., 2007a, Weese *et al*., 2012, Eckholm *et al*., 2013).

Several studies have found antimicrobial treatment to be a risk factor for MRSP infection (Eckholm *et al*., 2013, Weese *et al*., 2012, Gronthal *et al*., 2014). Studies that have not detected an association between treatment and presence of MRSP include one study on prevalence of MRSP from skin and carriage sites of dogs after treatment (Beck *et al*., 2012) and a case-control risk factor study of MRSP infection in dogs and cats (Lehner *et al*., 2014). Lehner and co-workers suggested that lack of proven association could be due to strain-specific variation or the longer time window for antimicrobial therapy in the study; analysis of more recent antimicrobial therapy might have shown a stronger effect on MRSP selection as adaption to different niches may occur more rapidly than anticipated (Lehner *et al*., 2014). Both Lehner and Beck also suggested that as an alternative explanation, MRSP is as well adapted to canine skin as MSSP. If so, selective pressure is of no benefit for MRSP (Lehner *et al*., 2014, Beck *et al*., 2012).

However, as mentioned above, logical hypotheses and biological data on antimicrobial therapy selecting for resistant bacteria support a risk of antimicrobial therapy selecting for MRSP carriage and infection. The normal bacterial skin flora occupies microbial niches and inhibits colonization by invading organisms, and it has been shown that antimicrobial treatment can

enable survival and colonization of pathogenic bacteria resistant to the antimicrobial in question by suppressing part of this flora (Muller, 2001, Graffunder and Venezia, 2002, Ogeer-Gyles *et al.*, 2006c).

The risk for facilitating bacterial growth and thereby prolonging clinically apparent infections by prescribing antimicrobials should also not be overlooked. Beck and co-workers found that 62% of MRSP positive dogs with clinical bacterial pyoderma were still MRSP positive after clinical resolution. In addition, 28% of dogs with bacterial pyoderma that previously had tested negative for MRSP were found to be MRSP positive after antimicrobial treatment. The authors concluded that the selection pressure exerted by antimicrobial therapy facilitated colonization of MRSP in the previously MRSP negative dogs. Furthermore, the study results suggested that systemic treatment with an antimicrobial effective *in vitro* against MRSP is unlikely to be effective for decolonization therapy (Beck *et al*., 2012).

Systemic glucocorticoid therapy has also been suspected to predispose for MRSP carriage in dogs, although presence of skin disease might have influenced the association (Nienhoff *et al*., 2011). Chronic skin disorder has been reported to be a risk factor for acquisition of MRSP. Factors that may contribute to the detected association include long-term antimicrobial pressure and frequent veterinary visits, as well as the changes in the skin (Griffeth *et al*., 2008, Huerta *et al*., 2011).

Staphylococcus pseudintermedius carriage rates have been shown to be higher in dogs with atopic dermatitis compared to healthy individuals. Furthermore larger populations of *S. pseudintermedius* have been found in dogs with dermatitis, compared to clinically normal dogs (Bannoehr and Guardabassi, 2012, Fazakerley *et al*., 2009, Lloyd D.H. *et al*., 1991, Harvey RG, 1998). In human medicine, the risk of becoming colonised and carry MRSA on the skin, (and thereby the risk of spreading the bacteria to other individuals) has been shown to increase with such skin changes (Higaki *et al*., 1999, Gong *et al*., 2006).

As mentioned above, healthcare-associated infections with MRSP have been described, and hospitalization as well as surgical invention has been suspected to increase the risk of MRSP colonization or infection in dogs. Contamination of veterinary hospital environments, as well as of staff, with MRSP has been documented. Investigations have found MRSP isolates that were indistinguishable when compared by PFGE from dogs, cats and the hospital environment they have been visiting, suggesting nosocomial transmission (Zubeir *et al*., 2007, van Duijkeren *et al*., 2008).

In a study by Bergström and co-workers, MRSP was detected in the hospital environment and from hospitalized dogs treated surgically, but not

from healthy dogs visiting the same environment (Bergstrom *et al*., 2012). There was also a significant increase in the number of dogs carrying MRSP after hospitalization compared to admission. Antimicrobial treatment was considered to be a potential contributing factor, as well as surgical stress, anesthesia, and change in environment. Grönthal and co-workers described a nosocomial outbreak of MRSP in a small animal hospital. The cases of MRSP infections were spatially and temporally connected. All infections were surgical site infections or other infections which appeared after prolonged hospital treatment, and none of the MRSP positive patients had evidence of MRSP on admission. The cumulative number of days spent in the intensive care unit or in the surgery ward was associated with an increased risk of MRSP positivity in hospitalized dogs (Gronthal *et al*., 2014). It has been suggested that veterinary hospitals and practices play an important, or even central role in the dissemination of MRSP in the general dog population. It is possible that MRSP initially emerges as more of a healthcare associated pathogen than a community associated pathogen. As colonized and infected patients becomes more prevalent in the community, the risk of community-acquired MRSP increases. Preventative measures towards spread of MRSP in veterinary healthcare environments should therefore be considered a critical cornerstone in combating the increase of this multidrug resistant bacterium (van Duijkeren *et al*., 2011a, Guardabassi and Prescott, 2015, Guardabassi, 2012, Weese and van Duijkeren, 2010, Lehner *et al*., 2014).

Transmission from MRSP infected or MRSP colonized dogs to healthy contact dogs living in the same household has been described in two recent studies, of which one was a longitudinal study and the other a one-point prevalence study (Laarhoven *et al*., 2011, van Duijkeren *et al*., 2011b). Information on horizontal transmission of MSSP between adult dogs is also scarce. Puppies have been shown to become colonized with MSSP soon after birth as a result of vertical transmission from the dam, equivalent to the mother-to-infant transmission of *S. aureus* seen in humans (Saijonmaa-Koulumies and Lloyd, 2002, Paul *et al*., 2014). In the study by Paul et al, both long-term persistence of strains transmitted vertically, and frequent sharing of strains between offspring belonging to the same litter was shown (Paul *et al*., 2014).

2 Aims

The overall aims of this thesis were to gain knowledge of bacterial infections in dogs in Sweden with special reference to urinary tract infections, surgical site infections and their antimicrobial susceptibility patterns, and of carriage of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). The results were intended to aid in choice of antimicrobial treatment of canine urinary tract infections and surgical site infections, and in designing recommendations on prevention and control of carriage of MRSP.

The specific aims were to:

- Investigate the relative presence of bacterial pathogens in urinary tract infections and surgical site infections in dogs and factors that might influence the culture results.
- Investigate susceptibility of bacterial isolates from urinary tract infections and surgical site infections. Multidrug resistant phenotypes, extended spectrum cephalosporin resistance in Enterobacteriaceae, MRSP and methicillin-resistant *Staphylococcus aureus* (MRSA) were of particular interest.
- Investigate if dogs clinically infected with MRSP can become asymptomatic long-term carriers, and investigate factors that might influence length of such carriage.
- Investigate potential MRSP carriage in contact dogs within multidog households where clinical MRSP infection has been diagnosed in one or several dogs.
- Collect and evaluate data relevant for development of sampling strategies for asymptomatic MRSP carriage in dogs.

3 Materials and Methods

3.1 Summary of study design

- Paper I and II are observational studies, where samples from clinical infections were analysed.

- Paper III and IV are longitudinal prospective studies where dogs diagnosed with a methicillin-resistant *S. pseudintermedius* (MRSP) infection were sampled repeatedly for possible carriage of MRSP, regardless of whether clinical symptoms of infection were present or not.

3.2 Study material

3.2.1 Samples

In study I and II samples from clinical infections were analysed.

In study I urine samples collected from dogs with clinical signs of urinary tract infection submitted by attending veterinarians to SVA (National Veterinary Institute, Sweden) for culture and susceptibility testing during a study period of ten months (March-December 2009) were investigated. The samples originated from four small animal referral hospitals, ten small animal clinics and 115 mixed veterinary practices. In total 623 samples from as many individual dogs yielded positive pathogenic growth. Forty-eight additional positive samples from already included dogs were evaluated separately. The samples were submitted as urine sent in a sterile container (19%), bacterial

swabs dipped in urine (13%), pre-incubated cultures on dipslides (42%) or agar plates (26%).

In study II four animal referral hospitals and three small animal clinics sent samples from all detected SSI in canine patients at time of diagnosis to SVA for culture and susceptibility testing during three years (April 1, 2008 - April 29, 2010). In total 157 cases of SSI where the samples yielded positive bacterial growth from a wound infection clinically diagnosed within a month after surgery were included. All samples were submitted as bacterial swabs.

In study III and IV, dogs diagnosed with an MRSP infection were sampled repeatedly for investigation of carriage of MRSP, regardless of whether clinical symptoms of infection were present or not after time of inclusion sample. A selective enrichment culture aimed at detecting MRSP was used.

In study III, 31 dogs diagnosed with an MRSP infection were sampled with intervals of at least six weeks until two consecutive negative results were obtained or until at least five months after the inclusion sample. Five body sites were sampled using bacterial swabs: nostrils, pharynx, perineum, the corner of the mouth and, when present, wounds.

In study IV, 11 dogs from four unrelated multiple dog households where one or two dogs had been diagnosed with an MRSP infection were included. All dogs in each family group were sampled simultaneously with 1-7 months intervals, during 7 (one family group) or 12- 15 months (three family groups). Four body sites were sampled using bacterial swabs: pharynx, perineum, the corner of the mouth and, when present, wounds.

3.2.2 Sample analyses

In study I and II routine microbiological culture and susceptibility testing of the submitted sample material was performed (CLSI, 2008, G.R. Carter., 1990, Barrow, 1993, Quinn P.J., 2011). In study I, a screening for presence of Enterobacteriaceae resistant to extended-spectrum cephalosporins (ESC) was performed through additional culture of the submitted sample material on selective screening media.

In study III and IV, a selective enrichment culture for MRSP was used. In study III, isolates from the last positive sample occasion was compared to either the inclusion sample (n = 21) or the first sample collected after inclusion (n = 5) by pulsed-field gel electrophoresis (PFGE). In study IV, all cultured MRSP isolates were genotypically characterized and compared using whole genome sequencing

3.2.3 Collected information and data included in the analyses

In study I, information was collected from recordings in the laboratory database based on the submission form submitted to SVA together with the samples.

Data included in the multivariable logistic regression analyses of the results from the bacteriological culture and antimicrobial susceptibility testing were: whether the samples were sent in as pre-incubated cultures or not, whether the cultures were contaminated or pure, and if the samples originated from referral hospitals, small animal clinics or mixed veterinary practices.

In study II, information was collected from a questionnaire completed by the attending clinician at time of sampling. Data included in the multivariable logistic regression analyses of the results from the bacteriological examination and antimicrobial susceptibility testing were: submission origin (which animal hospital or from a small animal clinic), duration of hospitalization, antimicrobial treatment, category of surgical procedures (clean, clean-contaminated, contaminated or dirty), depth of infection (superficial skin infections or deeper), and whether the culture was mixed with two pathogens or not.

In study III and IV, information was collected from a questionnaire completed by the attending clinician at time of sampling, as well as from the included dogs' medical records.

Data compiled and included in the multivariable logistic regression analyses of MRSP carriage over time were in study III: gender (female or male), age (≤ 6 years or older), diagnosis at time of clinical infection *i.e.* of the inclusion sample (dermatitis, surgical site infection, or infection/trauma), presence of dermatitis or wounds during the study, and time of systemic antimicrobial treatment. Data on dermatitis and presence of wounds were also included in evaluation of the relative number of MRSP positive cultures yielded from each sample site. Data on contacts with other dogs was compiled but not included in the analyses.

In study IV information on medical treatments, veterinary visits, skin lesions or wounds and symptoms of infection including dermatitis, as well as contacts with dogs outside of the household was collected.

4 Results and discussion

4.1 Bacterial growth in urinary tract infections and surgical site infections

4.1.1 Bacterial growth in the urinary samples

Seven different urinary pathogens were identified in 623 positive cultures with a specific urinary pathogen. *Escherichia coli* was the most prevalent, identified in 429 (68.9%) of the samples, followed by *S. pseudintermedius* (9.6%), *P. mirabilis* (8.8%), beta haemolytic *Streptococcus* spp. (5.6%), *Enterococcus* spp. (3.7%), *Klebsiella* spp. (1.8%) and *S. aureus* (1.6%).

Forty-eight positive cultures were repeated samples from dogs already included in the study. The same pathogen was isolated on both occasions for all of the dogs. The bacteria isolated were *E. coli* (81.2 %), *S. pseudintermedius* (6.3%), *P. mirabilis* (6.3%), *Klebsiella* spp. (4.2%), and *Enterococcus* spp. (2.0%).

The number of samples excluded due to either no growth, insignificant non-specific growth or growth of contaminants that prohibited further confirmation was 338 (35%) of the 1042 samples submitted.

There was a higher probability of finding *S. pseudintermedius* in pre-incubated samples (n = 49) compared to non-incubated samples (n=20) (OR=2.2; P = 0.019). The ten *S. aureus* isolates were all found in pre-incubated samples. The probability of finding *E. coli* was lower (OR=0.6; P=0.005) in contaminated cultures (n = 155) than in pure cultures (n = 274).

No association was found between the three submission categories (referral hospitals, small animal clinics or mixed veterinary practices) and relative prevalence of the respective bacterial species.

4.1.2 Investigation of influences on the culture and susceptibility results of the urinary tract infections

The seven urinary pathogens isolated in study I are the bacterial pathogens most frequently associated with UTI in dogs, and *E. coli* being the most prevalent with staphylococci in second place is also reflected in previous studies (Seguin *et al*., 2003, Bartges, 2004, Ling *et al*., 2001, Ogeer-Gyles *et al*., 2006a).

A positive culture with known bacterial pathogens was an inclusion criteria and a prerequisite for the diagnosis of UTI. Samples that yielded either no growth or only insignificant non-specific growth where therefore not further investigated in either study I or II, and it was the relative prevalence of each detected bacterial pathogen within the subgroup positive cultures that was investigated, not percentage positive cultures of all submitted samples.

Cystocentesis was the recommended method for collection of urine samples for bacteriological culture from SVA to all veterinarians submitting samples during the study period. However, approximately 40% of all samples were contaminated. Contamination of the urine samples indicate that other methods of sample collection were common. This was true also for samples from referral hospitals, known to have resources for cystocentesis and implementation of routines for collection of urine samples. As contaminated samples were common, the relative prevalence of the different pathogens was compared between contaminated and pure growth, both of which indicates the quality of collection- and handling procedures of the individual sample. For all but one bacterial species, there was no significant difference in prevalence between these two categories. The exception was *E. coli,* which was found significantly more often in pure culture, which indicates that the relatively high prevalence of *E. coli* in the material was not due to misinterpretation of contaminated samples.

To investigate the effect of sampling method and subsequent handling of samples including transport and transport medium on number of positive cultures (the susceptibility of the testing), a different study setup is needed, and the possible influence of collection method and handling of the samples on bacterial growth in study I could not be fully evaluated. The information was not included in the submission forms and although many of the referring veterinary clinics and hospitals had in-house recommendations for how to

collect and handle samples it was not possible to retrospectively retain reliable data regarding to which extent those recommendations had been followed. A retrospective investigation to clarify sample methods during the writing of study I was aborted as the information was decided not to become rigorous enough for statistical purposes. However, altogether including the results of the investigation of associations between findings of the isolated uropathogens, submission and if cultures were pure or contaminated - as well as investigation of associations between submission categories, the material was considered to be representative enough for describing bacterial growth and investigating antimicrobial susceptibility in UTI in the included dog population.

Choice of submitted sample material was not shown to be a major factor influencing the relative prevalence of bacterial species, with the exception of staphylococci, which were found more often in pre-incubated sample media. This finding supports the use of preincubated media when submitting urinary samples, as it lessens the risk of UTI infections with staphylococci, including MRSP, to go unnoticed.

4.1.3 Bacterial growth in the surgical site infections

In 37 (23%) of the positive cultures two pathogens were detected, leaving in total 194 pathogenic isolates from the 157 dogs included in the study. Eight different bacterial species were identified. *Staphylococcus pseudintermedius* was the most prevalent finding (46%), followed by beta haemolytic *Streptococcus* spp. (24%), *E. coli* (11%) and *S. aureus* (8%). The remaining pathogenic isolates were *S. schleiferi* (4%), *Pasteurella multocida* (3%), *Proteus mirabilis* (2%) and *Pseudomonas aeruginosa* (2%).

An additional pathogen was identified in 66% of the 47 cultures positive for beta haemolytic *Streptococcus* spp., and in 30% of the 90 cultures positive for *S. pseudintermedius.* It was significantly more common to find beta haemolytic *Streptococcus* spp. than S. *pseudintermedius, S. aureus, E. coli* and *Pseudomonas aeruginosa* in the 37 mixed cultures ($P<0.05$).

There were no associations between distribution of the isolated pathogens and submission category, duration of hospitalization or classification of the surgical procedure. Furthermore, there were no associations between any of the explanatory factors and whether there were two pathogens in the culture or not. One significant association between isolated pathogen and depth of infection was found. *Escherichia coli* was found significantly more often in deep wound infections than in superficial skin infections ($OR = 4.7$, $P = 0.02$). The reason for this finding is unknown.

4.1.4 Investigation of influences on the culture and susceptibility results of the surgical site infections

As in study I, attending veterinarians made the initial clinical diagnosis and collected all samples included in study II. A written description of standardised sample methods was sent to the enrolled hospitals and clinics together with the submission form and a questionnaire. All cases were sampled with a bacterial swab. A positive culture with known bacterial pathogens was an inclusion criterion, and a prerequisite for the diagnosis SSI. During the study period, 23% of 203 submitted samples were excluded due to the submitted samples yielding either no growth or only insignificant non-specific growth.

All the bacteria cultured in the study, including those less commonly associated with skin disease and skin carriage, might be endogenous flora recently transferred to the skin at the incision site through the dogs grooming behaviour, or through contamination of affected tissues during surgery (Nelson, 2011, Weese, 2013, May, 2006, Priestnall and Erles, 2011, Turk *et al*., 2015, Nicoll *et al*., 2014, Summers *et al*., 2012, Rodrigues Hoffmann *et al*., 2014, Quinn P.J., 2011, Eugster *et al*., 2004). Due to the possibility of prolonged contact with the microbiota in the healthcare environment influencing pathogenic growth, duration of hospitalization as well as submission category was recorded, but no influence on distribution of the eight bacterial species was shown (Wieler *et al*., 2011b, Bratzler *et al*., 2005, Nelson, 2011, Johnson, 2002).

As mentioned above, when evaluating SSI, an objective definition is desirable. This facilitates comparisons between veterinary studies, in which various definitions have been used for postoperative SSI (Weese, 2008c, Eugster *et al*., 2004, Nelson, 2011). In study II, The National Nosocomial Infections Surveillance criteria were used (Mangram *et al*., 1999). However, the standard classification of the wounds such as superficial incisional, deep incisional or organ/space was deemed not appropriate as, with the exception of the infections classified as superficial, the information attained from the attending veterinarians was decided not to be rigorous enough (Mangram *et al*., 1999). Infections including soft- or skeletal tissue or infections in the form of an abscess could have been classified as deep incisional, but as only seven isolates originated from this category with four bacterial species represented, the use of only the two terms superficial skin infection and deeper infection was deemed more stringent.

The National Nosocomial Infections Surveillance criteria were developed for surveillance of SSI in humans, and although a clear correlation between the four categories of surgical procedures and SSI rates has been reported in human medicine, it is not possible to determine either the risk of SSI to

develop or the source of the bacteria causing the SSI solely based on category of surgery (Mangram *et al*., 1999, Weese, 2008c, Eugster *et al*., 2004). However, the risk of an SSI being caused by an infecting agent present prior to surgery is increased in the dirty category, to which 18% of the 194 isolates in study II belonged. Furthermore, the risk of the SSI being caused by endogenous flora from the gastrointestinal, genitourinary or respiratory tracts can be expected to be lower in the clean category, as procedures where these body sites are opened are not included in this category (Mangram *et al*., 1999). No such correlations were found in study III. The only significant association between distribution of the isolated pathogen and the investigated explanatory factors was between growth of *E. coli* and depth of infection as well as with antimicrobial treatment during time of sampling. The reasons for these findings are unknown. The multifactorial nature of SSI, including critical factors such as adherence to aseptic principles and proper aseptic preparation and postoperative care in veterinary care facilities as well as in the home environment, complicates studies of this kind. Further studies on associations between individual bacterial species causing SSI and factors that can influence these are thus warranted.

4.1.5 Influence of antimicrobial treatment on the culture and susceptibility results

It has previously been stated that, as referral hospitals are more likely to have a higher caseload of complicated and recurrent medical and surgical cases, isolates can be expected to be less susceptible in samples originating from referral animal hospitals compared to smaller clinics. The samples included in study I and II were from veterinary practices with a wide geographical spread over the country and with both primary care facilities and referral animal hospitals represented. The material was thus considered representative of the actual dog population in the country. Interestingly, the resistance in UTI isolates was higher in samples originating from small animal clinics or mixed veterinary practices than in samples from referral animal hospitals.

Possible associations between submission category and antimicrobial susceptibility patterns could not be statistically evaluated in the SSI cases, due to the small number of isolates with diverging resistance patterns within each bacterial species.

In study I, previous and ongoing antimicrobial treatment was unknown. According to the Swedish Veterinary Association national guidelines for the clinical use of antibiotics in the treatment of dogs and cats (www.svf.se), culture and antimicrobial susceptibility testing should always be performed

when UTI is suspected in a dog. An exception is uncomplicated first-time UTI in young bitches. It is still possible that some dogs included in the study were sampled only after one or several antimicrobial treatments. However, the low percentage of resampled dogs correlates with previous reports of numbers of recurrent UTI (Ling, 1984, Norris *et al*., 2000, Seguin *et al*., 2003).

In study II, assessment of the influence of antimicrobial treatment on culture and resistance was not possible as too few isolates of each bacterial species were from cases that had received antimicrobial treatment, and even fewer from cases that had received treatment for more than one day (not only perioperative treatment). Notably, only twenty or less isolates were resistant to the agent used, which indicates that antimicrobial selective pressure on pathogenic growth was not significant.

4.2 Antimicrobial susceptibility patterns in urinary tract infections and surgical site infections

4.2.1 Antimicrobial susceptibility patterns in the urinary tract infections

There were some differences in antimicrobial susceptibility patterns in the repeated urinary samples compared to the first sample from each dog. In six of the seven cultures yielding resistant *E. coli*, the isolates were less susceptible in the repeated sample than on the first sample occasion. Four isolates were multidrug resistant (10% of the 39 *E. coli* isolates). Multidrug resistance was present in the first isolates from the three dogs involved, but the isolates from the repeated samples had additional resistance to trimethoprim-sulphamethoxazole. In one dog, an *S. pseudintermedius* isolate susceptible to penicillin was isolated on the first, and a penicillin resistant *S. pseudintermedius* isolate on the second sample occasion.

Comparison of susceptibility patterns between the three submission categories showed that the probability of finding trimethoprim-sulphamethoxazole resistant *E. coli* was higher from small animal clinics or mixed veterinary practices than from referral hospitals (OR=3.4; P=0.01 and OR=3.9; P=0.004, respectively). The probability of finding tetracycline resistant *E. coli* was higher (OR=2.5; P=0.04) for mixed veterinary practices than for referral hospitals. Furthermore, *E. coli* from mixed veterinary practices were more likely to be multidrug resistant than isolates from referral hospitals (OR=2.7; P=0.02).

Table 1. Antimicrobial susceptibility (% susceptible isolates) of *E. coli*, *Klebsiella* spp. and *P. mirabilis* in non-repeated urinary samples. The number of samples is shown inside brackets.

Table 1.

Antimicrobial	*Escherichia coli* (n=429)	*Klebsiella* spp. (n =11)	*Proteus mirabilis* (n=55)
	% S	% S	% S
Ampicillin	87.9	0.0	90.9
Amoxicillin/ Clavulanic acid	87.2	72.7	94.5
Cefotaxime	99.8	100	98.2
Gentamicin	94.9	90.9	83.6
Enrofloxacin	97.9	90.9	96.4
Tetracycline	92.1	100	5.5
Trimethoprim/ Sulphametoxazole	92.3	90.9	89.1
Nitrofurantoin	98.4	27.3	9.1

Table 2. Antimicrobial susceptibility (% susceptible isolates) of *S. pseudintermedius*, *S. aureus*, beta haemolytic streptococci and enterococci in non-repeated urinary samples. The number of samples is shown inside brackets.

Table 2.

Antimicrobial	*Staphylococcus pseudintermedius* (n=60)	*Staphylococcus aureus* (n=10)	Beta-hemolytic streptococci (n=33)	Enterococci (n=55)
	% S	% S	% S	% S
Penicillin	10.0	30.0	100	NR
Ampicillin	10.0	30.0	NR	87.0
Amoxicillin /Clavulanic acid	96.7	100	100	82.6
Cephalothin	98.3	100	100	13.0
Oxacillin	NR[a]	100	-	-
Gentamicin	98.3	100	0.0	30.4
Erythromycin	86.7	100	NR	34.8
Enrofloxacin	98.3	100	15.2	47.8
Tetracycline	66.7	90.0	63.6	65.2
Trimethoprim/ Sulphametoxazole	95.0	100	100	87.0
Nitrofurantoin	100	100	100	82.6

aNot relevant (NR) as breakpoints used to define isolates as susceptible (BP-S) is below the range of concentrations tested.

4.2.2 Antimicrobial susceptibility patterns in the surgical site infections

The majority of the isolates (68%) were from cases that had not received antimicrobial treatment prior to sampling. Also, 68% of the 62 isolates in the treated group were susceptible to the antimicrobial used. Seventeen percent of all 194 isolates were from cases sampled during ongoing antimicrobial treatment.

Escherichia coli was significantly more often found in samples collected during antimicrobial treatment than the other pathogens (OR=4.7, P=0.002).

Table 3. Antimicrobial susceptibility of isolated pathogens in the surgical site infections presented as the percentage of susceptible isolates (%S).

Table 3

		Antimicrobials tested[a]										
		AMP	PEN	CEP	OXA	CTX	ERY	CLI	ENR	GEN	TET	T-S
Staphylococcus pseudintermedius n=90	% S	20.0	20.0	95.6	NR[b]	NR[c]	76.7	NR[b]	94.4	93.3	73.3	75.6
Staphylococcus aureus n=15	% S	20.0	20.0	100	100	NR[c]	100	NR[b]	100	100	93.3	93.3
Staphylococcus schleiferi coagulans n=7	% S	14.3	14.3	100	NR[b]	NR[c]	71.4	85.7	100	100	85.7	100
beta haemolytic *Streptococcus* spp. n=47	% S	NR[b]	100	100	NR[c]	NR[c]	NR[b]	NR[b]	17.0	0.0	66.0	100
Escherichia coli n=20	% S	NR[b]	NR[c]	5.0	NR[c]	95.0	NR[c]	NR[c]	100	100	80.0	90.0
Pasteurella multocida n=6	% S	NR[c]	100	100	NR[c]	NR[c]	NR[c]	NR[c]	100	100	100	100

aAmpicillin (AMP), penicillin (PEN), cephalothin (CEP), oxacillin (OXA), cefotaxime (CTX), erythromycin (ERY), clindamycin (CLI), enrofloxacin (ENR),gentamicin (GEN),tetracycline (TET),trimethoprim-sulfamethoxazole(T-S).

bNot relevant (NR) as breakpoints used to define isolates as susceptible (BP-S) is below the range of concentrations tested.

cNot relevant (NR) as no BP-S is available

4.2.3 Multidrug resistance, ESC-resistance, detection of MRSA and MRSP in the urinary tract infections and surgical site infections

Multidrugresistance

Overall, multidrug resistance was rare, and all MDR isolates were susceptible to at least one antimicrobial relevant for the species and for the treatment of the investigated infections.

An MDR pathogen was detected in approximately 4% of all UTI cases, and in 19% of all SSI cases.

MDR was most common in *S. pseudintermedius*, as 10% (n=6) and 26% (n=23) of the isolates were MDR in study I and II, respectively. Approximately 4% (n=17) of *E. coli* isolates in study I were MDR. In study II, none of the in total 21 *E. coli* isolates were MDR.

One of the *P. mirabilis* isolates (2%) and two enterococcus isolates (9%) were MDR in study I, and in study II one beta-haemolytic *Streptococcus* spp. isolate had an MDR phenotype.

Resistance to extended-spectrum cephalosporins

Two isolates with a transferable gene conferring ESC resistance were detected in study I and II. In study I, one *E.coli* isolate carried the $bla_{\text{CMY-2}}$ gene and in study II one *E. coli* isolate carried the $bla_{\text{CTX-M-1}}$ gene. In addition one *P. mirabilis* isolate from study I produced AmpC beta-lactamase but was not tested for genotype.

Four of the ESC-positive isolates from UTI samples were detected in the screening culture, but not in the routine culture; two *E. coli* isolates and two *Enterobacter* isolates (*Enterobacter cloacae* and *Enterobacter aerogenes*). The two latter were most likely classified as contaminants in the routine culture of the samples. One *P. mirabilis* isolate diagnosed in routine culture was not detected on the screening agar. The small number of isolates preclude statistical assessment of potential benefits of the additional use of the selective screening culture for detection Enterobacteriaceae resistant to ESC in canine urinary samples.

Methicillin-resistant Staphylococcus aureus

No MRSA isolates were detected.

Methicillin-resistant Staphylococcus intermedius

Less than 3% (n=4) of *S. pseudintermedius* isolates in study I and II were confirmed as *mec*A carriers; 2% of the 60 *S. pseudintermedius* isolates in study I and 1% of the 90 *S. pseudintermedius* isolates in study II.

4.2.4 Interpretation of the antimicrobial susceptibility results from the investigated urinary tract infections and surgical site infections

In both studies, antimicrobial susceptibility was tested according to the standards of the Clinical and Laboratory Standards Institute (CLSI), using CLSI clinical minimum inhibitory concentrations (MIC) breakpoints (BPs) (CLSI, 2008). Although interpretive criteria might change, the use of these methods and breakpoints facilitates comparison of the results to future study results, and the results can provide a basis for future monitoring of antimicrobial resistance. The concentration ranges of the test panels used (VetMIC, SVA, Uppsala, Sweden) did not allow interpretation of MICs by CLSI BPs for all combinations of antibacterial and bacterial species. Only isolates susceptible according to CLSI BPs were included in the susceptible category, and when MDR was evaluated, all intermediately susceptible isolates were included in the resistant category.

Nitrofurantoin, gentamicin, and the combination trimethoprim-sulfamethoxazole were not intended to represent resistance towards other clinically relevant agents used for dogs. Furthermore, susceptibility to cefotaxime was tested as an indicator of ESC-resistance in *E. coli, Klebsiella* spp. and *P. mirabilis* isolates, although licensed products for dogs are available, for which the susceptibility testing is relevant (third-generation cephalosporins). Oxacillin was included as an indicator for methicillin resistance in staphylococci. Enrofloxacin was used as a representative for fluoroquinolones commonly used in dogs, although not for more recently developed fluoroquinolones such as pradofloxacine. Tetracycline susceptibility results were regarded as representative also for doxycycline.

Erythromycin has been used for treatment of staphylococcal pyoderma in dogs. It is also considered a relevant choice for treatment of intestinal infections with *Campylobacter spp*., and as a possible second choice in for example infections caused by anaerobic bacteria in dogs (Frank and Loeffler, 2012, Giguère *et al*., 2006, Noli and Boothe, 1990). Erythromycin is currently not approved for use in dogs in Sweden, and it was not intended to represent other macrolides, some of which are mainly used in large animal practice. However, the proportion of clindamycin susceptible staphylococcal isolates

and erythromycin susceptible staphylococcal isolates can be expected to be similar.

Clindamycin is a first-line antimicrobial for treatment of for example staphylococcal pyoderma (Giguère *et al*., 2006, Hillier *et al*., 2014). The CLSI BPs for clindamycin were outside of concentration ranges of the test panels used. However, although resistance in staphylococci can develop to lincosamides alone, lincosamid resistant strains are generally resistant to macrolides as well. The so called MLS-, or MLSB resistance phenotype (macrolide, lincosamide and streptogramin group B antibiotics resistance) can be either constitutive resistance, where bacteria show high-level resistance to all MLS antimicrobials, or inducible cross-resistance, in which the bacteria are resistant to macrolides but initially fully susceptible to clindamycin. Routine antimicrobial susceptibility testing of clindamycin can detect constitutive MLS resistance but fails to detect inducible resistance. Treatment failure can be expected when clindamycin is used for these strains. Simultaneous susceptibility testing to erythromycin is an aid in detecting such inducible clindamycin resistance, that should be suspected in isolates that *in vitro* are erythromycin resistant but clindamycin susceptible (Cain, 2013, Gold and Lawhon, 2013, Gortel, 2013).

The results of the susceptibility testing to benzylpenicillin **(**penicillin G) are also applicable to phenoxymethyl-penicillin (penicillin V). In a clinical situation this is also the case for the results of ampicillin and amoxicillin. Cephalotin was used as a representative for all first generation cephalosporins.

All staphylococci were tested for beta-lactamase- (penicillinase) production which, if present, renders the isolates resistant to penicillin, ampicillin and amoxicillin (aminopenicillins). Staphylococcal isolates susceptible to the tested cephalosporins and resistant to aminopenicillins through beta-lactamaseproduction, were considered to most likely be susceptible to the combination amoxicillin-clavulanic acid, as the clavulanic acid inactivates the beta-lactam enzymes.

In this thesis, the definition for phenotypic susceptibility testing of MDR recommended by Schwarz and co-workers was utilized; acquired resistance to three or more classes of antimicrobial agents (Schwarz *et al*., 2010). As described above, intermediately susceptible isolates were also included in the resistance category.

For the classification of MDR in *E. coli*, *Klebsiella* spp., and *P*. mirabilis isolated from UTI samples, ampicillin and amoxicillin-clavulanic acid were considered one antimicrobial class. For the classification of staphylococci, streptococci and enterococci, ampicillin, amoxicillin-clavulanic acid, cephalothin and penicillin were considered one antimicrobial class.

Antimicrobials not included in the classification of MDR due to inherently low susceptibility of the bacterial species included: for *Klebsiella* spp.; ampicillin and nitrofurantoin, for *P. mirabilis*; nitrofurantoin and tetracycline, and for streptococci enrofloxacin and gentamicin. Enterococci have constitutively low susceptibility to several of the antimicrobials studied, and only ampicillin, tetracycline, trimethoprim-sulfamethoxazole and nitrofurantoin were considered in the evaluation of MDR in this species (Kristich *et al*., 2014, Ossiprandi *et al*., 2008, Hollenbeck and Rice, 2012).

4.2.5 Antimicrobial treatment choices for treatment of the urinary tract infections and surgical site infections, a summary

Excluding the 4% MDR infections where still at least one relevant antimicrobial relevant for the bacterial species and respective infection was available, there were several relevant first-line treatment options in all the investigated UTI and SSI.

Susceptibility to first-line antimicrobials for UTI caused by *E. coli* was approximately 90-100%. In approximately half (56%) of all UTI, the causative pathogen was *E. coli* isolates susceptible to all the tested antimicrobials intended for clinical use (ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfadiazine, tetracycline, enrofloxacin, and nitrofurantoin).

Susceptibility rates were high also in the ten per cent caused by either *P. mirabilis* or *Klebsiella* spp. Susceptibility to penicillin and ampicillin was uncommon in the six percent of UTI caused by *S. pseudintermedius*. However, as susceptibility rates to amoxicillin-clavulanic acid, enrofloxacin, and nitrofurantoin were high not only in staphylococcal isolates but also in beta-haemolytic streptococci, and enterococci, several relevant treatment options were available also in the UTI caused by a gram-positive pathogen.

Excluding the 23% of the SSI cases where an MDR *S. pseudintermedius* isolate, including MRSP was detected, several first-line antimicrobials were available for treatment of the SSI. This includes cases where more than one pathogen was detected. Except for beta-lactamase resistance to aminopenicillins in staphylococci, the isolated pathogens from the SSI cases were mostly without acquired resistance.

- *Escherichia coli*

Susceptibility to first-line antimicrobials for *E.coli* infections in UTI was approximately 90-100%. Susceptibility rates for *E. coli* isolated from SSI cases were 80% to tetracycline and 90-100% for trimethoprim-sulfadiazine, tetracycline and enrofloxacin.

Except for treatment of UTI, a low susceptibility to ampicillin and first generation cephalosporins is expected for *E.coli*, which is reflected in only 5% of the SSI isolates being susceptible to first generation cephalosporins. Although the BPs for ampicillin were outside of concentration ranges of the test panels used for the SSI cases, the high ampicillin MIC_{50} indicates a large proportion of ampicillin resistant isolates.

Antimicrobials either not tested, or results not shown because the breakpoints used to define isolates as susceptible (BP-S) were below the range of concentrations tested, include penicillin, erythromycin and clindamycin, substances that are not expected to have an acceptable effect on *E. coli* infections.

- Staphylococci

In Sweden, resistance due to beta-lactamase production has during the last 14 years been recorded in between 75 and 90% of studied canine *S. pseudintermedius* isolates (SWEDRES-SVARM, 2013). Susceptibility to penicillin and ampicillin was, as expected due to beta-lactamase production, also uncommon in staphylococci in the investigated UTI and SSI. Only 10-20% of *S. pseudintermedius*, 20-30% of *S. aureus* isolates and 15% of the *S. schleiferi coagulans* isolates were susceptible.

For staphylococci in study I, susceptibility to amoxicillin-clavulanic acid was determined according to CLSI breakpoints for treatment of UTI, and susceptibility was high; 97-100%. For the SSI isolates, the CLSI BPs for this antimicrobial were outside of the concentration ranges of the test panels used. However, as the staphylococci were resistant to penicillin and ampicillin through beta-lactamase production, amoxicillin-clavulanic acid would most likely be a relevant antimicrobial for the isolates susceptible to cephalothin; 96% of the *S. pseudintermedius* isolates, 100% of the *S. aureus* and *S. schleiferi coagulans* isolates, respectively. Furthermore, an almost identical percentage of susceptibility to cephalothin was seen in staphylococcal isolates from the investigated SSI; 97% of the *S. pseudintermedius* isolates and 100% of the *S. aureus* isolates.

Although CLSI BPs for clindamycin were outside of concentration ranges of the test panels used for the SSI cases, erythromycin susceptibility was 77%, 100 % and 71 % for *S. pseudintermedius*, *S. aureus* and *S. schleiferi* respectively, indicating a similar proportion of clindamycin susceptible isolates, as discussed above.

Susceptibility rates to tetracycline were compared to the other antimicrobials tested lower; 67-75% in *S. pseudintermedius* isolates.

All staphylococcal isolates cultured from UTI cases were susceptible to nitrofurantoin.

- Beta-haemolytic streptococci

Beta-haemolytic streptococci were uniformly susceptible to penicillin. They are thereby also considered susceptible to ampicillin (Giguère *et al.*, 2006). The isolates were also uniformly susceptible to amoxicillin-clavulanic acid, and to trimethoprim-sulphametoxazole. Furthermore, the susceptibility rate to nitrofurantoin was 96-100%.

CLSI BPs were outside of concentration ranges of the test panels used for clindamycin. However, only one isolate had clindamycin MICs above the BP for resistance, and the MIC_{90} values were calculated for the isolates from SSI. These results indicate that most isolates in study II were susceptible to clindamycin.

The proportion of isolates susceptible to tetracycline was 64-66% and the inherently low susceptibility to enrofloxacin was reflected in the results, as only 15% of isolates were susceptible.

- Enterococci

More than 80% of all enterococci were susceptible to ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulphametoxazole and nitrofurantoin. In addition, approximately half of the isolates were susceptible to enrofloxacin.

The low susceptibility (30%) to the tested cephalosporin was expected, as enterococci express low-affinity penicillin-binding proteins (Kristich *et al.*, 2014).

4.3 Carriage of methicillin-resistant *Staphylococcus pseudintermedius*

4.3.1 Length of MRSP carriage

The repeated sampling of the 31 dogs enrolled in study III showed that dogs, after having had a clinically evident infection caused by MRSP, can carry the bacteria for several months without clinical signs. Conversely, some dogs were found to become MRSP negative within a year.

The overall median length of MRSP carriage in study III was 11 months. Five of the 21 dogs were shown to be MRSP positive for more than 14 months. Nine dogs were sampled for 5-12 months and remained positive. One of these dogs had a negative result on one sample occasion followed by positive results. Fifteen dogs were negative within 12 months and they remained negative 3.5 to 7.5 months later. Two dogs left the study at five and ten months, respectively, with negative results from all sample sites at the final sample occasion.

Twelve of the fifteen dogs in study III that were sampled until two consecutive negative results were achieved were found to be MRSP negative within nine months from the time of the inclusion sample, the remaining three within twelve months. Four of these dogs were MRSP negative on their very first sample occasion. According to the four dogs' medical charts, the inclusion sample was the first bacteriological sampling made in connection with the relevant diagnosis, and it is possible that the finding of MRSP in the inclusion sample of these dogs was a transient contamination.

Long-term carriage was also detected in study IV. Two of four dogs in study IV were MRSP positive when sampled for carriage four months after the inclusion sample, but negative on the next sampling occasion at 10 and 11 months. A third dog was still positive after 13 months.

It is possible that MRSP carriage in dogs is not always detectable. If so, a previously MRSP positive, then declared MRSP negative dog might become culture-positive again, for example due to positive selection of systemic antimicrobial treatment to which the bacteria is resistant. As guidelines regarding the possibility of declaring a dog as no longer carrying MRSP are lacking, a definition of probable MRSP negativity had to be decided prior to study III. Length of MRSP carriage was defined as time from the inclusion sample until the first of two consecutive negative sample occasions, with the exception of two dogs that were found to be negative on only one sample occasion, after which they left the study. These two dogs were regarded as MRSP negative on that last sample occasion.

Although the median time of carriage (11 months) in study III is useful for example when comparing results with future studies, it should be remembered that the exact length of carriage is difficult to determine. It is unknown if, and if so for how long, dogs were already carrying MRSP before the inclusion sample. Furthermore the period between sample occasions varied depending on when the dogs were available for sampling.

Intermittent carriage with reinfection of the sampled dogs cannot be ruled out. In addition to contact with other MRSP colonized dogs, humans and a contaminated environment might serve as a source of infection and reinfection (van Duijkeren *et al*., 2011b, Laarhoven *et al*., 2011). As the dogs were sampled at a veterinary clinic or hospital, there was also an opportunity for transmission of MRSP via contaminated environment or staff (Bergstrom *et al*., 2012, Gronthal *et al*., 2014, van Duijkeren *et al*., 2011b).

The samples in study III and IV were with few exceptions, including the index samples, not from a clinically infected site. Compared to many common bacterial samples from an infected body site, an increased risk of not being able to detect the bacteria of interest due to overgrowth of other bacteria and fungi might be present when sampling a carriage site. Furthermore, a low bacterial count could be a potential problem.

There were only two exceptions from consistently MRSP positive culture results from the sampled dogs. This indicates a low risk of false-negative results due to handling of samples and laboratory methods. In study III one of 27 dogs with at least one positive MRSP culture (index samples excluded) was negative on one sample occasion, but positive on the next. In study IV, the number was one of four contact dogs, and none of the four index dogs. Other longitudinal studies of MRSP in dogs have also noted such exceptions from consistently MRSP positive culture results (Bergstrom *et al*., 2012, Laarhoven *et al*., 2011).

A selective enrichment broth and a selective agar were used in both study III and IV to reduce the possibility of a false negative culture result (Laarhoven *et al*., 2011, Weese and van Duijkeren, 2010, van Duijkeren *et al*., 2011a). Multiple body sites were sampled to increase detection sensitivity (Weese and van Duijkeren, 2010, Laarhoven *et al*., 2011, Rubin and Chirino-Trejo, 2011). As a written description of standardised sample methods was sent to the veterinarians sampling the dogs, and due to the nature of the sampling, the standardisation could be expected to be easy to follow with one possible exception. In study III, where the nose was included as a sample site, several veterinarians found the nostrils to be more difficult to sample correctly than the other sample sites.

4.3.2 Investigation of factors that might influence length of MRSP carriage

Systemic treatment for three weeks or longer with antimicrobials to which the bacterium was resistant was in study III associated with prolonged carriage compared to shorter treatment periods. Three of five dogs treated with an antimicrobial to which their MRSP isolates were susceptible (tetracycline) were still MRSP positive after the end of treatment. No significant difference was found in study III between length of carriage and diagnosis at time of inclusion (dermatitis, surgical procedures or infection/trauma), age (≤ 6 years or older), gender (male or female), presence of wounds or signs of dermatitis during the longitudinal sampling. However, the presence of non-purulent wounds significantly increased the number of positive sample sites.

Although further research on the influence of antimicrobial treatment on MRSP carriage is warranted, logical hypotheses and biological data support the finding that systemic treatment with antimicrobials to which the cultured MRSP bacteria were resistant increased the length of detectable MRSP carriage. The results can be added to those of published studies that have found antimicrobial treatment to be a risk factor for MRSP infection. Dogs receiving systemic treatment with antimicrobials to which the bacterium is resistant may therefore have a higher risk of carrying, spreading, and developing clinical infections with MRSP. The risk for facilitating bacterial growth and thereby prolonging clinically apparent infections by prescribing antimicrobials should not be overlooked.

The finding that three of five dogs receiving systemic treatment with an antimicrobial to which their cultured MRSP isolates were susceptible remained MRSP positive was interpreted as a suggestion of MRSP having established itself as a part of the normal microbiota, which would prevent total eradication. Later publications have suggested that MRSP could be as well adapted to canine skin as MSSP (Lehner *et al*., 2014, Beck *et al*., 2012). Lehner and co-workers also suggested that one of several possible explanations behind their lack of proven association between antimicrobial treatment and MRSP positivity in dogs could be strain-specific variation (Lehner *et al*., 2014).

The finding that neither presence of wounds nor signs of dermatitis influenced length of carriage, and that dermatitis was not associated with an increased number of positive sample sites is interesting. In human medicine, the risk of becoming colonised and carry MRSA on the skin, (and thereby the risk of spreading the bacteria to other individuals) has been shown to increase with such skin changes (Higaki *et al*., 1999, Gong *et al*., 2006). The finding that presence of non-purulent wounds increased the number of positive sample sites (even when the wound as a sample site was excluded) is also interesting, as this could be interpreted as a higher risk of spread of MRSP to the

environment and other dogs. The sample size of respective group was however relatively small, resulting in a corresponding limitation in statistical power, and further studies with larger sample sizes might provide more information. This was also the case for another factor that was not shown to influence length of carriage: diagnosis at time of inclusion.

Data on medical treatment, veterinary visits, skin lesions or wounds and symptoms of infection including dermatitis, was also collected in study IV. However, the relatively small sample size precluded statistical analyses of whether presence of any of these factors were associated with difference in length of MRSP carriage or number of positive sample sites.

4.3.3 MRSP carriage in contact dogs

Table 4. Overview of MRSP positive (+) and negative (0) sample results, for dogs in the four family groups sampled in study IV, labelled A, B, C and D, respectively. Dogs are identified by family group and as index dogs (i for the first index dog in a group and ii for the second) or contact dogs (c or cc). Time of each sampling is shown in brackets as the number of months after inclusion sample.

Table 4.

	Sampling 1	Sampling 2	Sampling 3	Sampling 4
Family group A				
Ai	+	+	0	0
Aii	0	0	0	0
Ac	0	0	0	0
Acc	+	0	0	0
Time of sampling	*(1 month)*	*(4 months)*	*(11 months)*	*(12 months)*
Family group B				
Bi	+	+	+	+
Bc	0	0	0	0
Bcc	+	+	0	+
Time of sampling	*(1 month)*	*(4 months)*	*(10 months)*	*(13 months)*
Family group C				
Ci	+	+	0	0
Cc	+	0	0	0
Time of sampling	*(1 month)*	*(4 months)*	*(10 months)*	*(15 months)*
Family group D				
Di	+	+	0	Not sampled
Dc	+	+	0	Not sampled
Time of sampling	*(1 month)*	*(2 months)*	*(7 months)*	

The results of study IV support that there is a risk of transmission of MRSP between dogs living in households where MRSP infection has been diagnosed in one of the dogs. This is also reflected in the results of two other studies on multidog households (Laarhoven *et al*., 2011, van Duijkeren *et al*., 2011b).

As contact dogs only were found to be MRSP positive in combination with MRSP positivity in the index dog on the same sample occasion, the results of this study also indicate that the risk of MRSP colonization in dogs living in a household together with an MRSP infected dog is lowered if the index dog becomes MRSP negative. Furthermore, the finding that three contact dogs were consistently negative indicates that not all contact dogs carry MRSP continuously while living in a household where MRSP is present. One other published longitudinal study involved repeated cultures of contact animals within seven households where a dog was known to be MRSP–positive at inclusion time (Laarhoven *et al*., 2011). The authors of that study showed similar results in that MRSP positive contact animals generally were only found in combination with MRSP positive index dogs, except in one household where the index dog became MRSP negative while the contact animal was repeatedly MRSP positive. Furthermore, in a one point MRSP prevalence study by van Duijkeren and co-workers, contact animals were only MRSP positive if the index case showed clinical signs of infection at time of sampling, with the exception of one of 20 investigated households (van Duijkeren *et al*., 2011b).

Further longitudinal studies on MRSP carriage and transmission are warranted. However, the findings in study IV support the inclusion of risk of transmission of MRSP within multi dog households when developing infection control measurements. The possibility of not all contact dogs becoming long-term carriers should also be taken into account both in further studies on MRSP carriage and when recommending restrictions regarding contacts between dogs aiming at lowering the risk of further spread of MRSP from an MRSP positive household in the community.

4.3.4 Sample site evaluation

A positive MRSP culture was yielded simultaneously from all the five sample sites evaluated in study III (the corner of the mouth, nostrils, pharynx, perineum, and when present also wounds) on only 12% of the in total 73 positive sample occasions. On approximately one third (29%) of the positive sample occasions MRSP was yielded from only one of all the sampled sites

Wound was the sample site with the highest positive MRSP yield in study III, with MRSP detected on 81% (n=13) of 16 positive samplings of dogs with

wounds present (inclusion samples excluded). The bacteria were isolated from pharynx, perineum and the corner of the mouth in 67%, 63% and 58%, respectively, of the positive sample occasions. The nostrils were found to be the most difficult site to sample correctly and had the lowest positive yield (38%). Whenever the culture from the nostrils was MRSP positive, MRSP could also be found in one or more of the other sites.

The results show that simultaneous sampling of several body sites when screening clinically healthy dogs for MRSP should be recommended. A minimum of both a nasal and rectal or perineal swabs has previously been recommended for both MRSP and MSSP screening (Rubin and Chirino-Trejo, 2011, Weese and van Duijkeren, 2010). Although wound was the sample site with the highest positive MRSP yield, a negative wound culture should not be used as a definitive criterion for a dog being MRSP negative, as almost 20% of the wound samples were negative despite the bacteria being found in cultures from other sites that were sampled simultaneously.

The results suggest that the nostrils are not a priority when screening dogs for MRSP. Others have also suggested that, in contrast to *S. aureus* in humans, where nasal swabs are routinely used for screening purposes, canine carriers of *S. pseudintermedius* can most reliably be identified by swabbing both the oral mucosa and the perineum (Bannoehr and Guardabassi, 2012).

In study IV, the three sample sites used (wounds when present, perineum, and a pooled sample from pharynx and the corner of the mouth), were chosen based on the results from study III as well as on results from previous publications. Although there were relatively few MRSP positive dogs in study IV, the results supported the use of multiple sample sites. Notably more than one body site yielded a positive MRSP culture on only two of the fifteen positive sample occasions. Wound again had the highest MRSP positive yield: 71% (n=4) of the seven positive sample occasions where wound were present. Approximately half of the MRSP positive cultures, (45%; n=9) were from perineum. A third (30%; n=6) were from pharynx and the corner of the mouth.

4.3.5 Summary and possible sample strategies for detecting MRSP carriage

For strategic sampling for asymptomatic MRSP carriage, the recommended time for the first sample occasion could be shortly after either end of clinical signs of infection or the first MRSP positive carrier sample, to exclude contamination. If as few sample occasions as possible are important for financial reasons, a repeated sample after a period of at least nine months, preferably a year can be recommended. The owner needs to be informed of a

possible risk of MRSP not being detected, as well as that a previously declared MRSP negative dog might become culture-positive again.

The results suggest that simultaneous sampling of pharynx, perineum and the corner of the mouth, as well as wounds when present, should be recommended, and that sampling of nostrils is not a priority when screening dogs for MRSP.

Temporarily relocating contact dogs from a household where an MRSP infected dog has been detected might render repeated MRSP negative results in a shorter time period, compared to if the dogs had stayed together with the index case. Removing an MRSP positive dog temporarily from the household to avoid transmission to other pets has previously been suggested as a possible intervention (van Duijkeren *et al*., 2011b, van Duijkeren *et al*., 2011a). Other suggested preventative measures include bathing of the dog as this should reduce the contamination of the coat, and cleaning and disinfection of the contaminated environment with the aim to reduce the number of organisms (van Duijkeren *et al*., 2011a). Furthermore, proper basic hand hygiene routines in persons in contact with dogs have been suggested, with the aim to reduce transmission of coagulase-positive bacteria including MRSP between humans and pets in the household (Hanselman *et al*., 2009).

Systemic treatment with antimicrobials to which the bacterium is resistant may increase the risk, and length of, carriage and clinical infection with MRSP. Treatment with antimicrobials to which the bacteria is susceptible has not been shown to end MRSP carriage (Weese *et al*., 2012, Beck *et al*., 2012, Lehner *et al.*, 2014, Eckholm *et al*., 2013, Windahl *et al.*, 2012). Systemic antimicrobial treatment in dogs with possible or confirmed MRSP carriage or infection should therefore be avoided when possible.

It is possible that MRSP is emerging as more of a healthcare associated pathogen than a community associated pathogen. Veterinary hospitals and practices may play an important, or even central role in the dissemination of MRSP. When colonized and infected patients due to HAI become more prevalent in the community, the risk of community-acquired MRSP in turn increases. Preventative measures towards spread of MRSP in veterinary healthcare facilities should therefore be considered to be a cornerstone in combating the increase of this multidrug resistant bacterium (van Duijkeren *et al*., 2011a, Bergstrom *et al*., 2012, Wieler *et al*., 2011b).

5 Conclusions

- *Escherichia coli* was the most frequent pathogen in the investigated UTI, identified in approximately 70% of the cases. *Staphylococcus pseudintermedius* and *S. aureus* were more prevalent in pre-incubated samples than in non-incubated samples.

- Approximately two-thirds of all isolates identified in the investigated SSI were staphylococci. *Staphylococcus pseudintermedius* was the most frequent pathogen identified (46% of isolates). There were no associations between relative presence of bacterial species and category of surgical procedure (clean, clean-contaminated, contaminated or dirty).

- An MDR pathogen was detected in 4% of all UTI and in 19% of all SSI. Three percent of detected *E. coli* isolates were ESC-resistant. Less than 3% of *S. pseudintermedius* isolates were methicillin resistant. No MRSA isolates were found.

- First-line antimicrobials were found to be a rational empirical antimicrobial therapy for the studied dog population. Excluding the MDR infections where still at least one relevant antimicrobial was available, there were several relevant first-line treatment options in all the investigated UTI and SSI.

- Dogs carried MRSP for several months without clinical signs. Systemic treatment for three weeks or longer with antimicrobials to which the bacterium was resistant was associated with prolonged carriage compared to shorter treatment periods. Three of five dogs treated with an antimicrobial to which the MRSP isolates were susceptible remained MRSP

carriers. These findings support restricted use of systemic antimicrobial treatment in dogs with possible or confirmed MRSP carriage or infection.

- The risk of MRSP colonization in dogs living in a household with an MRSP infected dog might be lowered if the clinically infected dog (index dog) becomes MRSP negative. Furthermore, all contact dogs in the family might not carry MRSP continuously during the time the index dog is MRSP positive.

- The results of the evaluation of five body sites for MRSP carriage screening suggest that simultaneous sampling of pharynx, perineum and the corner of the mouth, as well as wounds when present, should be recommended. Furthermore, the results suggest that sampling of nostrils is not a priority when screening dogs for MRSP.

- For strategic sampling for asymptomatic MRSP carriage, the recommended time of first sample occasion could be shortly after either end of clinical signs of infection or the first MRSP positive carrier sample, to exclude contamination. If as few sample occasions as possible are important for financial reasons, a repeated sample after a period of at least nine months, preferably a year can be recommended.

6 Future perspectives

Several research- and knowledge gaps are readily identified. A few examples are listed below.

Neither the true prevalence of zoonotic MDR bacteria in dog populations, nor the actual zoonotic risk is well described. Further research into transmission of MRSP, Enterobacteriaceae with ESC-resistance and other potentially MDR bacteria between dogs, as well as between dogs and humans is needed.

Published surveillance reports on antimicrobial resistance in dogs are currently few, and much baseline data on antimicrobial susceptibility needed for clinical therapy decisions, as well as for development of policy recommendations for companion animals, is lacking. An increased internationally coordinated surveillance of antimicrobial resistance in canine bacteria would be beneficial, also to permit the early detection of resistant strains and support investigation of outbreaks. Importantly, such surveillance should include not only cultures from patients that are "worst case scenarios", but a broad, representative population so that the actual levels of antimicrobial resistance are reflected. Relative bacterial growth, and susceptibility patterns in specified infections such as UTI and SSI, should be presented so that studies and reports can be compared. Such reports also aid in evaluation of in-house surveillance schemes at individual animal hospitals and clinics, including recognizing diverging results so that appropriate measures are instigated.

Preventive measures against infections in both individual animals and broader animal populations that influence animal health and/or public health should be a priority in veterinary medicine. Surprisingly little attention has in the world of dog breeding been directed towards the possibility of breeding dogs that are less prone to skin disease and secondary bacterial skin infections including pyoderma. An increase in research directed towards how to breed dogs less at risk for developing such bacterial infections is warranted.

Further research is needed in the area of underlying factors for bacterial disease, included pathogenic mechanisms of key pathogens such as *S. pseudintermedius* and MRSP. Development of effective vaccines and other evidence based preventative strategies, including more veterinary-specific products aimed at treatment of key infections and key pathogens would be most welcome in small-animal medicine

Based on current knowledge, relevant measures in reducing spread of bacteria in the dog population include health care infection control and rational antimicrobial use. However, much more knowledge is needed, including in the area of community-associated epidemiology. Veterinarians as well as dog owners only have a limited amount of research-based information when deciding whether a dog, or a group of dogs, should be considered high- or low-risk individuals regarding transmission of bacteria to other dogs, or to humans. Further knowledge is also needed on what interventions should be prioritized, and how the effect of such interventions should be measured. Further longitudinal studies of carriage of MRSP and MRSA are clearly warranted.

The potentially potent role of dogs as part of planned treatment strategies in human healthcare has recently been increasingly acknowledged. Research based guidelines on how to best prevent possible risks for zoonotic bacterial carriage or infections due to such human-pet interactions is much needed.

Furthermore, the possible role of dogs in transmission and outbreaks of infections with various zoonotic bacteria to and from food producing animals is poorly investigated.

Increasing knowledge and awareness of the value of diagnostic investigation, and prudent antimicrobial use is needed both in the veterinary field and the general public. Widely, easy accessible research-based best practice manuals, including information directed at the animal-owners could support attending veterinarians when trying to change old patterns in use of antimicrobials. An increased knowledge and awareness of the value of implementing proper infection control, and on how to establish infection control programs is needed in the veterinary community.

7 Populärvetenskaplig sammanfattning

En ökad förekomst av antibiotikaresistenta bakterier hos hund rapporteras från hela världen. Både den behandlande veterinären och djurägaren ställs i och med detta idag allt oftare inför svåra val. Infektioner som tidigare framgångsrikt kunde behandlas med antibiotika kan idag leda till att hunden avlivas. Antibiotikabehandling med antibiotika som bakterien är resistent mot kan ge förvärrade infektionssymtom.

Utdragna infektionsförlopp leder till ett ökat lidande för den enskilda hunden. Varje enskild infektion med resistenta bakterier kan också innebära en risk för ytterligare spridning av bakterien eller resistensen. Bakterier med ESC-resistens (resistens mot extended-spectrum cephalosporins), MRSA (meticillin-resistenta *Staphylococcus aureus*), samt MRSP (meticillin-resistenta *Staphylococcus pseudintermedius*), är exempel på särskilt problematiska infektioner. ESC-resistens kan spridas inte bara genom smitta av resistenta bakterier mellan människor, människa-hund och hundar, utan också genom att de gener som bär på resistensen överförs mellan olika bakteriearter. MRSA infektioner har hittills varit mycket ovanliga hos hund jämfört med hos människa, där den i många länder orsakar stora problem.

Bakterien *Staphylococcus pseudintermedius* (*S. pseudintermedius*) är en del av hundens normala bakterieflora, men samtidigt också den i särklass viktigaste och vanligaste orsaken till bakteriella infektioner hos hund. Sällsynta fall av infektion hos andra djur, och hos människa med denna bakterie har rapporterats, men anses vara ovanliga. År 2005 uppmäksammades de första fallen i Europa av en multiresistent variant av denna bakterie, MRSP. MRSP infektioner hos svenska hundar har som regel varit resistenta mot samtliga antibiotika i tablett- och injektionsform registrerade för hund, med ett undantag: tetracyklin. En del infektioner har även varit resistenta mot detta antibiotikum.

En snabb ökning av antalet MRSP fall har samtidigt påvisats i både Europa och Nordamerika. Mycket kunskap som behövs för att ta fram relevanta åtgärdsplaner mot fortsatt spridning, och för hur man bör provta hundar som kanske kan sprida smittan utan att de själva har symtom saknas.

I de två första studierna i denna av handling undersöktes bakterier som orsakade urinvägsinfektioner och infektioner i operationssår, och vilka antibiotika de var resistenta mot. *Escherichia coli* (*E. coli*) var den vanligaste bakterien i urinvägsinfektionerna. Den allra vanligaste bakterien i sårinfektionerna var *S. pseudintermedius*, som påvisades i nära hälften (46 %) av alla sårinfektioner. Tillsammans påvisades tre typer av stafylokocker, inkluderande *S. pseudintermedius*, i ungefär 60 % av sårinfektionerna.

Undersökning av bakterie växten i de olika provmaterial som användes till urinproverna visade att stafylokocker kan vara lättare att hitta om den provtagande veterinären förodlar provet innan det skickas vidare till ett annat laboratorium för vidare undersökning. För sårinfektionerna gick det inte att se någon statistisk skillnad mellan vilka bakterier som orsakade infektion och vilken typ av operation som hade utförts.

Jämfört med många internationella rapporter var resistensen överlag låg. Äldre rekommendationer avseende förstahandsval av antibiotika till hund var fortfarande relevanta. I de allra flest fallen fanns ett flertal antibiotika registrerade för hund att välja på, och det fanns aldrig något behov av att använda två antibiotika samtidigt, eller antibiotika avsedda att i första hand användas till allvarliga infektioner hos människa.

Multiresistenta bakterier påvisades dock i cirka 4 % av totalt 623 stycken urinvägsinfektioner och 19 % av totalt 154 stycken sårinfektioner. I tre infektioner påvisades också bakterier med ESC-resistens, vilket för några år sedan inte setts hos svenska hundar. I fyra infektioner påvisades MRSP bakterier. Inga MRSA infektioner påvisades.

De två sista studierna inriktade sig på smittspridning av MRSP hos hundar. Resultaten visade att hundar kan bära på MRSP utan att visa symtom i över ett år, efter att den ursprungliga infektionen (till exempel hudinfektion eller sårinfektion) läkt av. Hos en del hundar kunde dock bakterien inte längre hittas redan efter några månader. Faktorer som skulle kunna påverka bärarskapets längd undersöktes, och behandling med antibiotika som bakterien var resistent mot visades förlänga tiden. Fem hundar fick antibiotika om bakterien var känslig för. Tre av dessa fortsatta att bära på MRSP. Dessa fynd stödjer undvikande av antibiotikabehandling av hundar som kan misstänkas bära på MRSP.

MRSP kunde inte alltid hittas hos övriga hundar i hushåll med en MRSP infekterad hund. Hundarna tenderade att bli MRSP negativa när den ursprungligen MRSP infekterade hunden blev negativ.

Fem provtagningsställen jämfördes, för att kunna ge rekommendationer om effektiv provtagning för bärarskap av MRSP. Resultaten visade att flera ställen bör provtas för att säkert hitta MRSP. Sår var säkrast, därefter området kring analöppningen samt mungipa/svalg.

Sammantaget rekommenderas att sår eller hudskada, samt både svalg, området kring analöppningen och mungipa provtas för MRSP hos symtomfria hundar. Första provtagningstillfället kan förläggas en kort tid efter att hunden är symtomfri, och om det är positivt upprepas efter nio månader eller längre tid, när så få provtagningstillfällen som möjligt önskas. En del hundar i samma hushåll som en MRSP infekterad hund kanske kan bli MRSP negativa snabbare om de byter miljö och inte träffar den infekterade hunden. Vidare forskning behövs dock innan man säkert kan säga hur stor risken är att en hund med MRSP negativa prover trots detta kan visa sig vara MRSP positiv en tid senare, till exempel efter en antibiotikabehandling med antibiotika som bakterien är resistent mot.

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