Epidemiology of methicillin-resistant
*Staphylococcus pseudintermedius* in
Finland

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ACADEMIC DISSERTATION

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Abstract

*Staphylococcus pseudintermedius* is part of the normal microbiota of dogs and cats. Since the mid-1980s, an ever-increasing number of methicillin-resistant *S. pseudintermedius* (MRSP) isolates have been reported. The situation worsened in the mid-2000s, when two predominant MRSP clones, ST71 (sequence type 71) and ST68, spread through Europe and North America, respectively. MRSP isolates are commonly multidrug resistant (MDR), and are thus capable of causing infections that do not respond to routinely used antimicrobials. MRSP appeared in the small animal population of Finland in the late 2000s, also causing numerous infections at the Veterinary Teaching Hospital (VTH) of the University of Helsinki. Finnish surveillance data also indicated that the proportion of MRSP among *S. pseudintermedius* isolates drastically increased in 2009–2011. MRSP has been under intense study worldwide in recent years, but no data on the epidemiology of the bacterium in Finland have been published.

This thesis study aimed to explore the epidemiology of MRSP in the Finnish small animal population, and to report antimicrobial susceptibility for *S. pseudintermedius*. This was done by investigating and describing the MRSP outbreak at the VTH, and investigating risk factors for patients being colonized or infected by MRSP in the hospital during the outbreak. The prevalence of MRSP and the risk factors for MRSP carriage were investigated in a canine subpopulation at the Guide Dog School for the Visually Impaired. The susceptibility of *S. pseudintermedius* isolates to antimicrobials in 2011–2015 was investigated using data from the Clinical Microbiology Laboratory (CML) of the Faculty of Veterinary Medicine, University of Helsinki. Risk factors for an *S. pseudintermedius* isolate being MRSP, as well as for a screening specimen revealing MRPS, were also investigated. Furthermore, the molecular epidemiology of all MRSP isolates stored in 2010–2014 was investigated using pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and staphylococcal cassette chromosome mec (SCCmec) typing.

Antimicrobial therapy, whether previous or ongoing during sampling, was identified as a risk factor for MRSP in all studies. Furthermore, a prolonged hospital stay and veterinary visits were identifiable risk factors among guide dogs. An *S. pseudintermedius* isolate originating from a private clinic (versus the VTH) was a significant risk factor for MRSP among clinical specimens. The same could be seen among screening specimens from patients with risk factors for MRSP. In addition, it was noted that a sizeable proportion (~20–60%) of animals in the studies had been or were being treated with antimicrobials.
During the outbreak at the VTH, rigorous hygiene and barrier measures were necessary to achieve control. ST71, the MRSP clone that caused the outbreak, was the predominant clone in 2010–2011, accounting for over 50% of MRSP isolates, even among non-outbreak-related isolates. By 2014, however, the situation had changed, as ST71 represented only ~10% of MRSP isolates. MRSP clones belonging to CC45 (clonal complex 45) and CC258, as well as a plethora of unrelated STs, dominated the MRSP population by that time. SCCmec type IV was detected in a majority of different STs, indicating the horizontal spread of resistance genes.

The prevalence of MRSP was only 3% among guide dogs. The proportion of clinical specimens from small animals that revealed MRSP was similar, being 2.5%. However, 9% of screening specimens from high-risk patients revealed MRSP. Overall, 14% of *S. pseudintermedius* isolates were MRSP. Roughly 30–40% of isolates were not susceptible to alternative antimicrobials, such as lincosamides, macrolides, or tetracyclines. MRSP in feline specimens was rare (<1% in both clinical and screening specimens after 2011).

Our results give further credence to the hypothesis that antimicrobial therapy and contact with the veterinary environment are risk factors for MRSP in small animals. However, cats do not appear to be a significant source of MRSP. Our data suggest that the epidemiology of MRSP has changed from a predominantly clonal spread to a mix of clonal spread and the spread of genetic elements. The resistance rates among *S. pseudintermedius* are at an alarming level. Decisive action, including the use of non-antimicrobial treatments whenever feasible and more prudent use of antimicrobials, is required to improve the situation.
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List of original publications

This thesis is based on the following publications:


The publications are referred to in the text by their Roman numerals, and are reproduced with the permission of the copyright holder. In addition, some unpublished material is presented.
Abbreviations

ATCC® American Type Culture Collection
bp Base pair
CA Community associated
CA-MRSA Community-associated methicillin-resistant *Staphylococcus aureus*
CA-MRSP Community-associated methicillin-resistant *Staphylococcus pseudintermedius*
CC Clonal complex
ccr Gene coding cassette chromosome recombinases
CI Confidence interval
CLI Clindamycin
CML Clinical Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Helsinki
CoNS Coagulase-negative staphylococci
CLSI Clinical and Laboratory Standards Institute
ERY Erythromycin
Evira Finnish Food Safety Authority
FiRe Finnish Study Group for Antimicrobial Resistance
FUS Fusidic acid
HA Healthcare associated
HA-MRSA Healthcare-associated methicillin-resistant *Staphylococcus aureus*
HA-MRSP Healthcare-associated methicillin-resistant *Staphylococcus pseudintermedius*
ICU Intensive care unit
LIS Laboratory information system
MDR Multidrug resistant
meca Gene conferring methicillin resistance by coding penicillin binding protein 2a (PBP2a)
MIC Minimum inhibitory concentration
MLSb Macrolide, lincosamide, streptogramin B
MLST Multi-locus sequence typing
MRSA Methicillin-resistant *Staphylococcus aureus*
MRSP Methicillin-resistant *Staphylococcus pseudintermedius*
MSSP Methicillin-susceptible *Staphylococcus pseudintermedius*
OR Odds ratio
OXA Oxacillin
PBP Penicillin binding protein
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PC</td>
<td>Private (veterinary) clinic</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>Polymerase chain reaction - restriction fragment length polymorphism</td>
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<tr>
<td>PFGE</td>
<td>Pulsed field gel electrophoresis</td>
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<tr>
<td>SCCmec</td>
<td>Staphylococcal cassette chromosome mec</td>
</tr>
<tr>
<td>SSI</td>
<td>Surgical site infection</td>
</tr>
<tr>
<td>ST</td>
<td>Sequence type</td>
</tr>
<tr>
<td>SXT</td>
<td>Sulfamethoxazole and trimethoprim</td>
</tr>
<tr>
<td>TET</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>TPLO</td>
<td>Tibial plateau leveling osteotomy</td>
</tr>
<tr>
<td>VTH</td>
<td>Veterinary Teaching Hospital of the University of Helsinki</td>
</tr>
<tr>
<td>WGS</td>
<td>Whole genome sequencing</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1 Introduction

*Staphylococcus intermedius* was originally discovered in 1976 (Hajek, 1976) and later renamed in 2005 (Devriese *et al.*, 2005) as *Staphylococcus pseudintermedius*. It is a significant opportunistic pathogen of small animals. It is frequently associated with dermatitis and otitis, as well as surgical site and urinary tract infections (Weese and van Duijkeren, 2010; Windahl *et al.*, 2015). Since the beginning of the 21st century, an increasing number of methicillin-resistant isolates (methicillin resistant *Staphylococcus pseudintermedius*, MRSP) have been discovered. The majority of MRSP isolates are multidrug resistant (MDR), and infections caused by these may be very difficult to treat due to the lack of effective antimicrobial agents (van Duijkeren *et al.*, 2011a). These bacteria also have the potential to spread in veterinary clinics and hospitals (Bergstrom *et al.*, 2012; Zubeir *et al.*, 2007). Due to the aforementioned factors, MRSP has an impact on the veterinary medical community, and the general public, by increasing treatment costs and compromising animal health and welfare. The spread of resistant bacteria may also impact on public health, as *S. pseudintermedius* can be spread between humans and animals (van Duijkeren *et al.*, 2008; van Duijkeren *et al.*, 2011b), and may cause infections in humans (Borjesson *et al.*, 2014; Starlander *et al.*, 2014). Additionally, the spread of resistance genes between different species of staphylococci, e.g. from *S. pseudintermedius* to *Staphylococcus aureus*, via mobile genetic elements has the potential to cause the proliferation of MRSA (Hanssen and Ericson Sollid, 2006).

This doctoral thesis explores the infection and molecular epidemiology of MRSP by investigating the antimicrobial resistance of Finnish *S. pseudintermedius* isolates, determining the prevalence of MRSP in Finnish guide dogs and patients with risk factors for MRSP, assessing the crude prevalence of MRSP based on results from clinical specimens, investigating factors that predispose to MRSP infection or colonization, and exploring the genetic relatedness of MRSP isolates in Finland.

To investigate susceptibility and molecular epidemiology, a collection of *S. pseudintermedius* and MRSP strains isolated in the Clinical Microbiology Laboratory (CML) of the Faculty of Veterinary Medicine at the University of Helsinki was examined. Risk factors were investigated using cross-sectional studies on an MRSP outbreak at the University of Helsinki Veterinary Teaching Hospital (VTH), data from the Guide Dog School of the Finnish Federation of the Visually Impaired, and laboratory data. The molecular epidemiology of MRSP was studied by pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and staphylococcal cassette chromosome *mec* (SCCmec) analyses.
2 Review of the literature

2.1 Staphylococcus pseudintermedius

Staphylococci are Gram-positive, catalase-positive cocci, and members of the Staphylococcacae family (Becker and von Eiff, 2011). These bacteria are common commensals of the skin and mucous membranes in mammals (including humans) and birds (Becker and von Eiff, 2011). *S. pseudintermedius* (previously identified and named as *S. intermedius*) is part of the normal microbiota of dogs and cats (Cox et al., 1985; Devriese and De Pelsmaecker, 1987; Devriese et al., 2005; van Duijkeren et al., 2011a), but is also an opportunistic pathogen. In general, cats carry *S. pseudintermedius* at a lower rate than do dogs (Cox et al., 1988; Cox et al., 1985; Lilenbaum et al., 1999; Lilenbaum et al., 1998). The bacterium is commonly associated with ear and skin infections in the dog and cat (Fitzgerald, 2009; Huerta et al., 2011; Kadlec et al., 2011; Kania et al., 2004). Staphylococcal infections are traditionally treated with first-generation cephalosporins or amoxicillin potentiated with clavulanic acid. Other treatment options include a combination of sulfonamides and trimethoprim, lincosamides such as clindamycin, or macrolides such as erythromycin and tylosin (Sykes, 2013). The susceptibility of staphylococci to these drugs has declined in previous years. One example of this is the rapidly increasing resistance to methicillin, which has led to fewer therapeutic alternatives (Perreten et al., 2010).

2.2 Taxonomy and identification of *S. pseudintermedius*

*S. pseudintermedius*, first described in 2005 by Devriese et al. (2005) and isolated from a cat, a dog, a horse, and a parrot (Devriese et al., 2005), is part of the *Staphylococcus intermedius* group (SIG). Two additional species, *Staphylococcus intermedius* and *Staphylococcus delphini*, also belong to this group. *S. intermedius* was initially identified in 1976 from pigeons, dogs, mink, and horses (Hajek, 1976). *S. delphini* was originally found in dolphins (Varaldo et al., 1988). The taxonomy of the SIG is presented in Figure 1.

Previously identified canine *S. intermedius* isolates have been reclassified as *S. pseudintermedius*, as identification based on *sodA* and *cpn60* sequencing allows the differentiation of SIG species (Bannoehr et al., 2007; Sasaki et al., 2007b). The current consensus is that canine isolates belonging to the SIG can be presumptively reported as *S. pseudintermedius* (Devriese et al., 2009). As such, it should be noted that canine *S. intermedius* isolates, particularly prior to 2005,
are probably *S. pseudintermedius* isolates. As a consequence of this, the abbreviation MRSI (methicillin-resistant *Staphylococcus intermedius*) has been replaced by the abbreviation MRSP. Furthermore, all *S. intermedius* findings from small animals will be presumed to be *S. pseudintermedius* for the purpose of this review, as was suggested by Devriese *et al.* (2009), and reported as *S. (pseud)intermedius*.

![The taxonomy of the *Staphylococcus intermedius* group](image)

**Figure 1** The taxonomy of the *Staphylococcus intermedius* group (gray area) and related bacteria. The scale indicates the % difference in average nucleotide identity. Simplified from Ben Zakour *et al.* (2012).

The biochemical differentiation of species belonging to SIG can be difficult. While *S. intermedius* and *S. pseudintermedius* can be differentiated, for example, by arginine dihydrolase and D-mannitol-tests, *S. delphini* and *S. pseudintermedius* cannot be reliably differentiated using biochemical reactions (Sasaki *et al.*, 2007b). Biochemical test kits, such as the ID32 Staph (bioMérieux, France), are also unable to differentiate the species within this group (Layer *et al.*, 2006; Sasaki *et al.*, 2007b).

However, a simple PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) method in which the *pta* (phosphate acetyltransferase) gene is first amplified and then restricted using an *MboI* restriction enzyme has been shown to be useful in differentiating *S. pseudintermedius* from other members of the SIG (Bannoehr *et al.*, 2009). Although SIG members are very similar, differentiation between them may also be achieved by 16S rRNA sequencing, the most common method for identifying bacterial species (Devriese *et al.*, 2005). Furthermore, mass spectroscopy, such as MALDI-TOF (Matrix Assisted Light Desorption Ionization - Time of Flight) has been studied as a tool for distinguishing the members of the SIG, with promising results (Decristophoris *et al.*, 2011).
In human medicine, the importance of *S. pseudintermedius* and other members of the SIG as pathogens may previously have been underestimated, as misidentification as *Staphylococcus aureus* is known to occur (Borjesson *et al.*, 2014). There is even a study reporting MSSP being misidentified as MRSA using common clinical laboratory techniques (Pottumarthy *et al.*, 2004). Lee *et al.* (2015) recently described an algorithm for identifying SIG members in a clinical laboratory setting by utilizing colony morphology, a latex agglutination test, DNase test, and VITEK 2.

### 2.3 Virulence factors

The infections caused by *S. pseudintermedius* and *S. aureus* are fairly similar, although the latter has been studied in greater detail (Weese and van Duijkeren, 2010). *S. pseudintermedius* is associated with a plethora of infections in companion animals, such as pyoderma, otitis, surgical wound infections, cystitis, metritis, and osteomyelitis (Cabassu and Moissonnier, 2007; Fitzgerald, 2009; Huerta *et al.*, 2011; Kania *et al.*, 2004; Ruscher *et al.*, 2009; Weese and van Duijkeren, 2010).

Some factors affecting the virulence of *S. pseudintermedius* have been identified. Features in common with *S. aureus*, such as coagulase, and many proteases and thermonucleases, have been discovered in *S. pseudintermedius* (Fitzgerald, 2009). *S. pseudintermedius* also produces toxins such as hemolysin (alpha and beta-hemolysin), exfoliative toxin and enterotoxins (Quinn *et al.*, 2011). Exfoliative toxin likely plays an important role in the development of staphylococcal pyoderma, as it can cause subcorneal clefts in the canine epidermis (Iyori *et al.*, 2011). Iyori *et al.* (2011) demonstrated that the exfoliative toxin producing *orf* gene was more common in *S. pseudintermedius* isolates from dogs with pyoderma compared to healthy dogs (23% vs. 6%). Also, Terauchi *et al.* (2003) isolated an exfoliative toxin-like toxin that showed strong cell rounding activity from *S. (pseud)intermedius* isolates derived from dogs with pyoderma. The classical virulence factor of *S. aureus*, Panton-Valentine Leucocidin (PVL), has not been detected in *S. pseudintermedius* (Bardiau *et al.*, 2013; Wedley *et al.*, 2014; Weese *et al.*, 2009). Leukotoxin production has nevertheless been discovered among canine *S. pseudintermedius* isolates (Futagawa-Saito *et al.*, 2004).

Furthermore, *S. pseudintermedius* has the ability to bind to fibrinogen, fibronectin, and cytokeratin (Geoghegan *et al.*, 2009), common microbial surface components recognizing adhesive matrix molecules found in *S. aureus* (Heilmann, 2011). *S. pseudintermedius* also carries an immunoglobulin-binding protein that resembles staphylococcal protein A (*spa*) found in *S. aureus* (Moodley *et al.*, 2009). In addition, the bacterium has the ability to form biofilm.
(Osland et al., 2012), a characteristic that results in increased tolerance to antimicrobials and is a well-established problem with infections involving orthopedic implants (Olsen, 2015). Furthermore, at least one study indicates that \textit{agr} (accessory gene regulator) gene homologues, which are involved in quorum sensing, have been detected in some \textit{S. (pseud)intermedius} isolates (Dufour et al., 2002).

2.4 Infection, colonization and contamination

As with any bacterium, an animal may be classified as having an infection, colonization or contamination by the bacterium. An infection implies that the bacterium has been able to avert the hosts’ primary immune response and has established an infection accompanied by an inflammatory response (redness, swelling, pain, warmth, and loss of function). This may be localized, e.g. an outer ear infection, or generalized, i.e. systemic.

Colonization is a state where the animal is a carrier of the bacterium for a prolonged time, i.e. the bacterium may be repeatedly isolated from subsequent specimens from the same animal. Colonization with \textit{S. pseudintermedius} is very common in dogs, as it is considered part of the normal microbiota (Cox et al., 1985; Devriese and De Pelsmaecker, 1987; van Duijkeren et al., 2011a). Colonization with methicillin-resistant \textit{Staphylococcus pseudintermedius} (MRSP) is less common (Beck et al., 2012), and there are indications that some dogs may eliminate MRSP over time (Windahl et al., 2012). Colonized animals are probably an important method of spread for MRSP (as they are for MRSA), as they do not show symptoms of disease (van Duijkeren et al., 2011a; Weese, 2010).

The third category, contamination, is a state whereby the animal is only a transient carrier of the bacterium. In these cases, the bacterium can usually be isolated only once from the animal, while subsequent specimens do not reveal it. It may be difficult to distinguish between colonization and contamination, as it is dependent upon factors such as the interval with which specimens are obtained.

2.5 Methicillin resistance in \textit{S. pseudintermedius}

MRSP, as well as most other methicillin-resistant staphylococci, carry the \textit{mecA} gene in their DNA (Ubukata et al., 1989). \textit{mecA} resides in a mobile genetic element, a staphylococcal cassette chromosome (SCC), designated SCC\textit{mec}, of which there are currently eleven (I–XI) different types (IWG-SCC, 2017b). The features of SCC\textit{mec} are further discussed in section 2.8.2. It is believed that \textit{mecA} originated in \textit{Staphylococcus scuiri}, one of the most primitive species of
staphylococci (Couto et al., 1996). Some *S. aureus* and coagulase-negative *Staphylococcus* sp. (CoNS) isolates carry a *mecA* homolog, *mecC*, which has thus far been reported to only be carried by SCCmec XI (Liu et al., 2016; Shore et al., 2011). Neither *mecC* nor SCCmec XI have yet been detected in *S. pseudintermedius*. The *mecA* gene codes for a specific penicillin binding protein (PBP2a) that methicillin and other beta-lactam-class drugs bind to ineffectively (Ubukata et al., 1989). In the bacterial cell, PBPs are responsible for the cross-linking of the glycopeptide polymer units in the peptidoglycan layer of the cell wall. Beta-lactams inhibit this cross-linking, causing defects in cell wall synthesis, which leads to the death of actively dividing cells (Prescott, 2013a). Due to the ineffective binding of beta-lactams to the altered PBPs, MRSP is resistant to this entire class of antimicrobials, except for ceftaroline, a 5th generation cephalosporin (Duplessis and Crum-Cianflone, 2011).

Using conventional methods, the identification of MRSP from a clinical specimen takes at least 2–3 days. Presumptive identification of MRSP is based on species identification and resistance to oxacillin, a methicillin analogue commonly used for the screening of methicillin-resistant staphylococci (MRS) (CLSI, 2013a, b). The use of a 10-μg oxacillin disk has been shown to predict methicillin resistance (i.e. the presence of *mecA*) in *S. pseudintermedius* very well when the zone of inhibition (ZOI) is ≤17 mm (Bemis et al., 2009). Cefoxitin, which is the main method to screen for methicillin resistance in *S. aureus*, is not a sensitive method to screen for *mecA* in *S. pseudintermedius* (Bemis et al., 2009; Bemis et al., 2012). Further testing is required to confirm the isolate as MRSP. This is most reliably done by PCR to detect the *mecA* gene (van Duijkeren et al., 2011a). This method may, however, not be available in some veterinary laboratories. An alternative method is the detection of the altered surface protein by a latex-based agglutination method. The sensitivity and specificity of latex agglutination tests have been shown to be high for *S. aureus* (Baddour et al., 2007; Nakatomi and Sugiyama, 1998). However, caution should be used when investigating atypical isolates that do not grow on routinely used testing media, as cefoxitin inducement of PBP2a production may be required (Miller et al., 2017). While agglutination tests are a useful tool to detect PBP2a in *S. aureus*, and have been used in studies regarding *S. pseudintermedius*, they have not been validated for MRSP. Indeed, at least one study has reported cases where MSSP isolates have given false-positive PBP2a latex agglutination results (Pottumarthy et al., 2004). It would be important to develop a cost-effective method for rapid and accurate MRSP detection, such as the tests developed for MRSA (Patel et al., 2015).

Infections caused by MRSP can be difficult to treat owing to the fact that it is also frequently resistant to a number of other antimicrobial classes (Borjesson et al., 2012; Perreten et al., 2010) (see section 2.6). In addition, MRSP can cause problems in veterinary hospitals and clinics, as it is capable of nosocomial spread.
The proportion of MRSP among *S. pseudintermedius* isolates and the prevalence of MRSP among animals varies between studies and countries, but both figures have increased in recent years. The occurrence of MRSP is further discussed in section 2.9.

### 2.6 Resistance to other antimicrobials

#### 2.6.1 Resistance to macrolides and lincosamides

Macrolides and lincosamides both inhibit protein synthesis in bacteria by binding to the 50S subunit of the ribosome (Giguère, 2013a, b). Macrolides inhibit the transpeptidation and translocation process, thus causing premature detachment of the polypeptide chain (Giguère, 2013b), while lincosamides inhibit peptidyl transferases (Giguère, 2013a). These mechanisms are very similar, and the ribosomal binding sites of these classes of drugs are the same or closely related (Giguère, 2013a). Macrolide drugs commonly used in dogs and cats include erythromycin, clarithromycin, azithromycin, and tylosin, while the most commonly used lincosamide is clindamycin (Sykes, 2013).

Resistance to macrolides and lincosamides is caused by the methylation of rRNA (most commonly), efflux mechanisms, or enzymatic inactivation (Giguère, 2013a, b). Cross-resistance between macrolides and lincosamides, as well as streptogramin B (also called MLSb), is common (Giguère, 2013a). This resistance is due to the methylation of the 23S ribosomal RNA (rRNA), which prevents the drug from binding to this site (Laing and Weisblum, 1971; Leclercq and Courvalin, 1991). Methylases are encoded by the *erm* gene family (Weisblum, 1995), of which the most common representative among MRSP isolates is *ermB* (Perreten et al., 2010). Cross-resistance among MLSb can be either constitutive, conferring resistance to the entire group, or dissociated inducible, where the bacteria appear susceptible to lincosamides, but rapidly develop resistance during treatment (Giguère, 2013a). This inducible resistance should be tested for during routine susceptibility testing (CLSI, 2013a; Gold and Lawhon, 2013).

Resistance to macrolides is prevalent among *S. pseudintermedius*, with resistant strains making up 9% to 28% of investigated isolates (Ganiere et al., 2005; Intorre et al., 2007; Norstrom et al., 2009; Rubin et al., 2011; Vanni et al., 2009; Windahl et al., 2015). Macrolide resistance among MRSP isolates is very common (87–100%) (Kjellman et al., 2015; Perreten et al., 2010; Ruscher et al., 2010; Sasaki et al., 2007a).
Due to the aforementioned cross-resistance, resistance to lincosamides among *S. pseudintermedius* is roughly as common as macrolide resistance, ranging from 10 to 22% (Ganiere *et al.*, 2005; Kawakami *et al.*, 2010; Vanni *et al.*, 2009), although one study found resistance to clindamycin as high as 99% (Casagrande Proietti *et al.*, 2012). Macrolides were not tested in that study. Resistance among MRSP isolates is in general significantly higher, being 87–100% (De Lucia *et al.*, 2011; Kawakami *et al.*, 2010; Kjellman *et al.*, 2015; Perreten *et al.*, 2010; Ruscher *et al.*, 2010; Sasaki *et al.*, 2007a). The prevalence of inducible clindamycin resistance among *S. pseudintermedius* isolates has been reported to be 5% (Chanchaithong and Prapasarakul, 2015). Inducible clindamycin resistance among MRSP isolates is rare, as it is far more common for MRSP to be resistant to both macrolides and lincosamides, i.e. constitutively resistant (Faires *et al.*, 2009).

2.6.2 Resistance to tetracyclines

Tetracyclines inhibit protein synthesis in the bacterial cell by binding to the 30S subunit of the ribosome (del Castillo, 2013). Resistance is most commonly mediated by either an efflux system or ribosomal protection proteins that detach tetracyclines from their binding sites (del Castillo, 2013; Roberts, 1996). The spectrum of resistance among tetracyclines is associated with the type of *tet* gene, the main mediator of tetracycline resistance. *tet*(K) confers resistance to tetracycline, but not to doxycycline and minocycline (Frank and Loeffler, 2012). *tet*(K) genes are common in the main European MRSP clone, ST71 (Perreten *et al.*, 2010). *tet*(M) genes, on the other hand, confer resistance to all mentioned tetracyclines (Frank and Loeffler, 2012). Differences in tetracycline and doxycycline resistance are probably due to the presence of either *tet*(M) or *tet*(K) (Ganiere *et al.*, 2005). More recent studies suggest that *tet*(M) has become an important mediator of resistance to tetracyclines among *S. pseudintermedius*, (Maaland *et al.*, 2013; Norstrom *et al.*, 2009).

Tetracycline resistance is quite common among *S. pseudintermedius* isolates, with percentages ranging from 24 to 46%, (Ganiere *et al.*, 2005; Rubin *et al.*, 2011; Windahl *et al.*, 2015). However, a study by Casagrande Proietti *et al.* (2012) revealed very high levels of resistance to doxycycline (96%), although the selection criteria for the investigated isolates were not disclosed. Resistance among MRSP isolates is generally higher (46–90%) (Casagrande Proietti *et al.*, 2012; Kjellman *et al.*, 2015; Perreten *et al.*, 2010).
2.6.3 Resistance to fluoroquinolones

Fluoroquinolones are bactericidal antimicrobials mainly used to treat infections caused by Gram-negative bacteria (Sykes, 2013). Their effect is based on their ability to cause defects in the negative supercoiling of DNA in the bacterial cell by targeting DNA topoisomerases (Giguère and Dowling, 2013). It has been found that different fluoroquinolones primarily target either topoisomerase IV or DNA gyrase (topoisomerase II), or both. Type I fluoroquinolones (e.g. ciprofloxacin, and levofloxacin) mainly target topoisomerase IV, type II fluoroquinolones (e.g. nadifloxacin) mainly target DNA gyrase, and type III fluoroquinolones (e.g. moxifloxacin and pradofloxacin) target both equally (dual-target property) (Takei et al., 2001; Wetzstein, 2005).

Fluoroquinolones commonly used in veterinary medicine primarily represent two different generations of the drug class, II and III (Giguère and Dowling, 2013). Enrofloxacin, representing generation II, is the most common fluoroquinolone in veterinary medicine, while pradofloxacin is the sole generation III fluoroquinolone approved for animal use (Giguère and Dowling, 2013). Pradofloxacin is more potent against S. pseudintermedius than enrofloxacin (Blondeau et al., 2012; Schink et al., 2013; Silley et al., 2012). This is probably because third-generation fluoroquinolones have a preferential affinity for topoisomerase IV as a primary target (Drlica and Malik, 2003), although pradofloxacin does display dual targeting of both topoisomerase IV and DNA gyrase (Wetzstein, 2005). Resistance to fluoroquinolones can be caused by target modification, decreased permeability, efflux, and target protection. These mechanisms are not mutually exclusive and may appear simultaneously (Giguère and Dowling, 2013). Mutations in the gyrA gene confer fluoroquinolone resistance in S. pseudintermedius (Intorre et al., 2007).

Reports regarding the susceptibility of S. pseudintermedius to enrofloxacin have reported resistance figures between 0% and 23% (Ganiere et al., 2005; Intorre et al., 2007; Lilenbaum et al., 1999; Rubin et al., 2011; Vanni et al., 2009; Windahl et al., 2015), while respective figures for MRSP are markedly higher, being 45–100% (De Lucia et al., 2011; Morris et al., 2006; Perreten et al., 2010). Kjellman et al. (2015) found that fluoroquinolone resistance was associated with MRSP ST71, as all representatives of that lineage displayed resistance to ciprofloxacin (enrofloxacin was not tested).

Pradofloxacin has thus far not been readily available for susceptibility testing, and epidemiological data regarding the prevalence of pradofloxacin resistance in S. pseudintermedius are therefore scarce (Ganiere et al., 2005; Kizerwetter-Swida et al., 2016). Extensive data on cross-resistance between enrofloxacin and pradofloxacin have not yet been published, although Ganiere et al. (2005) did find one S. pseudintermedius isolate that was resistant to enrofloxacin and marbofloxacin (MIC 16 mg/L), while the pradofloxacin MIC of the same isolate was only 1 mg/L. This result would be classified as intermediate (Kizerwetter-
Swida et al., 2016). Kizerwetter-Swida et al. (2016) found nearly complete (35/36 isolates) cross-resistance between enrofloxacin, ciprofloxacin and pradofloxacin.

2.6.4 Resistance to aminoglycosides
Aminoglycosides cause misreading of the genetic code by attaching to the 30S subunit of the bacterial ribosome, thus disrupting protein synthesis. These antimicrobials are mainly used to treat severe infections caused by Gram-negative aerobes and staphylococci (Dowling, 2013a), or in the topical therapy of outer ear infections, for example (Sykes, 2013). Aminoglycosides are bactericidal, but require parenteral dosing for a systemic effect. There is also concern about their nephrotoxic effects, limiting their use (Dowling, 2013a). The primary aminoglycoside used in small animals is gentamicin, although amikacin may also be used for systemic therapy (Papich, 2012; Sykes, 2013). Topical preparations may also contain other aminoglycosides, such as neomycin (Lääketietokeskus, 2016). Resistance to aminoglycoside among staphylococci is most often mediated by modifying enzymes (Ardic et al., 2006). Bacteria may simultaneously carry numerous different genes that encode diverse aminoglycoside-modifying enzymes (Ardic et al., 2006; Dowling, 2013a). In S. pseudintermedius, insertion sequence 256 (IS256) has been associated with resistance to gentamicin and amikacin (Casagrande Proietti et al., 2012). The resistance mechanism or type of enzyme produced determines the spectrum of resistance. In general, amikacin is more tolerant of inactivating enzymes (Dowling, 2013a).

Resistance to aminoglycosides has been described in several studies. Studies regarding gentamicin (the type drug for the group) have revealed that resistance among all S. pseudintermedius isolates range between 0% and 29% (Lilenbaum et al., 1999; Lilienbaum et al., 1998; Rubin et al., 2011; Vanni et al., 2009; Windahl et al., 2015; Youn et al., 2011). Among MRSP isolates, resistance to gentamicin has varied between 39% and 100% (Casagrande Proietti et al., 2012; Kjellman et al., 2015; Morris et al., 2006; Perreten et al., 2010; Ruscher et al., 2010; Sasaki et al., 2007a). Amikacin resistance is less reported among S. pseudintermedius, but according to a study by Gold et al. (2014), resistant isolates made up nearly 18% of studied isolates in 2012. On the other hand, an Italian study by De Lucia et al. (2011) found no resistance to the drug. Amikacin resistance among MRSP isolates appears to be more common (59%) (Casagrande Proietti et al., 2012).

2.6.5 Resistance to potentiated sulfonamides
Sulfonamides and diaminopyrimidines are combined to achieve a bactericidal effect by blocking consecutive steps in the folic acid synthesis pathway of bacteria (Prescott, 2013b). The combination of sulfamethoxazole and trimethoprim (SXT)
is often the one used in susceptibility testing (CLSI, 2013a, b). Sulfonamide—trimetoprim combinations are used for a number of different infections in dogs and cats (Prescott, 2013b; Sykes, 2013). In addition, these combinations have a wide margin of safety, although adverse effects particularly due to sulfonamides do occur. Resistance to SXT may be mediated though a number of mechanisms, including impairment of drug penetration, production of insensitive enzymes, or hyperproduction of PABA (Prescott, 2013b).

Resistance to SXT among S. pseudintermedius is low to moderate, with studies showing 5 to 30 percent of isolates being resistant (Beever et al., 2015; Ganiere et al., 2005; Humphries et al., 2016; Priyantha et al., 2016; Rubin et al., 2011; Windahl et al., 2015). However, high levels of resistance (60 to 97%) have been observed in some studies (Casagrande Proietti et al., 2012; Youn et al., 2011). MRSP isolates are generally more resistant to SXT, with 22% to 100% being resistant to the drug (De Lucia et al., 2011; Humphries et al., 2016; Kjellman et al., 2015; Sasaki et al., 2007a). Nevertheless, as a British study found, resistance levels can even vary immensely between laboratories in the same country (Beever et al., 2015). The testing of SXT can be technically difficult, as excess thymine in the testing media may lead to excessively high resistance results (CLSI, 2013a, b).

2.6.6 Resistance to miscellaneous antimicrobials

**Fusidic acid** is a lipophilic steroid antibiotic (Dowling, 2013c). In veterinary medicine, it is used as topical treatment for pyogranulomatous dermatitis (i.e. hot spot), as well as ear and eye infections (Clark et al., 2015; Finnish Food Safety Authority and the Faculty of Veterinary Medicine, 2016). Resistance to this compound has been less frequently studied, probably at least in part due to the lack of Food and Drug Administration (FDA) approved preparations in the United States of America (Dowling, 2013c). Additionally, while EUCAST has breakpoints for the interpretation of fusidic acid susceptibility, CLSI does not. This makes comparison of results more difficult. The Finnish Study Group for Antimicrobial Resistance (FiRe) standard used to have interpretation criteria for fusidic acid (10-μg disc, cut-off values: S ≥ 24 mm, R ≤ 18 mm), which were based on a study by Skov et al. (2001). These have now been replaced by the EUCAST standard (cut-off values for fusidic acid S ≥ 24, R < 24). Limited information is available also on the resistance mechanism to this drug, although alteration of the elongation factor of permeability and enzymatic inactivation have been implicated (Turnidge and Collignon, 1999).

Since the drug is primarily used for topical therapy, some studies, such as that by Loeffler et al. (2008) have reported fusidic acid as susceptible, even when MICs have been higher than the current EUCAST breakpoints. This seems appropriate, as the achievable drug concentrations are significantly higher.
topically than systemically. It would be inappropriate to directly compare results from different studies, as the criteria for susceptible and non-susceptible may differ. It is also important to note that no breakpoints have been defined for susceptibility testing for local therapy. Loeffler et al. (2008) investigated *S. pseudintermedius* and MRSP isolates from dogs and cats, and found that all isolates were probably susceptible to concentrations achievable by topical therapy (MIC$_{90}$ ≤ 4 mg/L). Vanni et al. (2009) made similar discoveries among *S. (pseud)intermedius* isolates from Italy. Likewise, Clark et al. (2015) found that nearly all *S. pseudintermedius* isolates, regardless of whether they were MRSP, were susceptible to fusidic acid concentrations that are achievable using local therapy (MIC < 16 mg/L). In an Italian study, De Lucia et al. (2011) did not find any fusidic acid resistance among MRSP isolates (n = 48). A Norwegian study determined that nearly half of all MRSP isolates were resistant to fusidic acid when using the ≥ 1 mg/L MIC breakpoint for resistance set by EUCAST (EUCAST, 2017; Norstrom et al., 2009). Available fusidic acid preparations in Finland contain 5 (otic) or 10 (ophthalmic) mg/g of fusidic acid (Lääketietokeskus, 2016), a concentration that far exceeds the breakpoint set by EUCAST (2017).

**Chloramphenicol**, a phenicol antimicrobial, like fusidic acid, is mainly used for topical therapy in cases of conjunctivitis (Sykes, 2013). It is rarely used systemically in veterinary medicine, but has been suggested as a treatment option for MRSP infections, where traditional antimicrobials are found ineffective (Frank and Loeffler, 2012; Papich, 2012). The antimicrobial works by binding irreversibly to the 50S subunit of the bacterial ribosome, inhibiting protein synthesis (Dowling, 2013b). Resistance to chloramphenicol is most commonly caused by enzymatic inactivation through acetylation (chloramphenicol acetyltransferase, CAT), which prevents the binding of the drug to the ribosomal subunit (Dowling, 2013b). One gene encoding for these enzymes, *cat_pC21*, has been described in representatives of the major European MRSP lineage, ST71 (Perreten et al., 2010). As such, MRSP isolates representing ST71 from Europe are generally not susceptible to chloramphenicol, but their ST68 North American counterparts usually are (Perreten et al., 2010). However, a Spanish study did not detect chloramphenicol resistance among ST71 isolates (Gomez-Sanz et al., 2011).

Chloramphenicol resistance among *S. pseudintermedius* is rather rare as a whole. Vanni et al. (2009) reported only 3% of isolates resistant to the drug, while Rubin et al. (2011) did not detect any resistance to it. Low resistance (0.9%) was also reported in a Canadian study (Priyantha et al., 2016). Among MRSP isolates, resistance rates are higher. While investigating the association of amikacin resistance to a number of antimicrobials Gold et al. (2014) found that 30% out of 422 investigated isolates were resistant to chloramphenicol. An Italian study reported that 60% of MRSP isolates (n = 48) were chloramphenicol resistant (De Lucia et al., 2011).
2.7 Screening for MRSP

As with MRSA carriers in humans (Senn et al., 2016; van Rijen et al., 2009), MRSP has the potential to spread via healthy carriers (Bergstrom et al., 2012; van Duijkeren et al., 2011b). In humans, MRSA is usually screened for by nasal, axillae, and perineal swabs, which are commonly enriched and then plated onto selective media (Dodemont et al., 2015; Micheel et al., 2015), or directly analyzed using PCR (Huletisky et al., 2004; Wassenberg et al., 2010). The sampling sites for MRSP screening in dogs vary somewhat in the literature, but particularly the nose, mouth, and perianal region/rectum appear in many. Windahl et al. (2012) collected specimens from the nostrils, corner of the mouth, perineum, and pharynx of dogs. The results indicated that not all sample sites were positive for MRSP, despite the animal being a carrier. They reported that the pharynx, closely followed by the perineum, yielded the most MRSP isolations. Beck et al. (2012) collected specimens from the nose, rectum, and affected skin, and Hanselman et al. (2008) from the nose, axilla, and rectum of dogs. The former study found MRSP from both nasal and rectal swabs at the same rate, while the latter only found MRSP from nasal swabs. The nose was the sole site sampled in a previous study of canine patients by Sasaki et al. (2007a). In a Swedish study, specimens were obtained from the mouth, nostril, and perianal region (Bergstrom et al., 2012). In addition to these, an Australian study also sampled the ear of the animal (Bean and Wigmore, 2016). S. (pseud)intermedius may be isolated from the nose, mouth, anus, vulva, and skin of dogs (Sajjonmaa-Kouluemies et al., 1998). Fewer studies have been conducted on cats, but in one study on cats entering a veterinary teaching hospital, the nose, pharynx, perineum, and possible skin lesions were swabbed to detect MRSP carriers (Nienhoff et al., 2011a).

Most studies in which MRSP screening has been performed have utilized an enrichment step to increase the sensitivity of the test (Beck et al., 2012; Gomez-Sanz et al., 2011; Hanselman et al., 2008; Laarhoven et al., 2011; Morris et al., 2010; Murphy et al., 2009; Paul et al., 2012; Paul et al., 2011; van Duijkeren et al., 2011b; Windahl et al., 2012). This enrichment step is performed either in Müller-Hinton (Laarhoven et al., 2011; Paul et al., 2012; Paul et al., 2011), tryptic soy (Bergstrom et al., 2012; van Duijkeren et al., 2011b; Windahl et al., 2012), or brain-heart infusion broth (Gomez-Sanz et al., 2011), and may or may not contain selective agents. Common selective agents include sodium chloride (4–7.5% NaCl) (Bergstrom et al., 2012; Gomez-Sanz et al., 2011; Hanselman et al., 2008; Laarhoven et al., 2011; Murphy et al., 2009; Paul et al., 2011; van Duijkeren et al., 2011b; Windahl et al., 2012), aztreonam (Bergstrom et al., 2012; Laarhoven et al., 2011; van Duijkeren et al., 2011b; Windahl et al., 2012), ceftizoxime (Laarhoven et al., 2011; van Duijkeren et al., 2011b), cefoxitin (Bergstrom et al., 2012; Windahl et al., 2012), or oxacillin (Morris et al., 2010).
No commercial media for the screening of MRSP exist. As commercial media for MRSA screening probably contain cefoxitin, a more sensitive predictor of MRSA (CLSI, 2013a; Pourmand et al., 2014), the media may contain too much of the antimicrobial to allow MRSP with lower MICs to grow. This may result in MRSP carriers being missed when using media designated for MRSA, to screen for MRSP. The agars used in published studies have often been non-selective if the enrichment medium contains antimicrobial agents (van Duijkeren et al., 2011b; Windahl et al., 2016; Windahl et al., 2012), and vice-versa (Beck et al., 2012; Gomez-Sanz et al., 2011; Laarhoven et al., 2011; Paul et al., 2011). However, some studies have used antimicrobials in both (Morris et al., 2010; Murphy et al., 2009). Commercial selective agars used include ORSAB (Oxoid) (Gomez-Sanz et al., 2011), MRSA Brilliance (Oxoid) (Paul et al., 2011), and BBL CHROMagar (Becton, Dickinson & Co.) (Beck et al., 2012). Mannitol-salt agar with oxacillin has also been used (Hanselman et al., 2008).

2.8 Molecular methods used for typing MRSP

2.8.1 Pulsed-field gel electrophoresis
Pulsed-field gel electrophoresis (PFGE), described in the mid 1980s (Schwartz and Cantor, 1984), is a method for determining the relatedness of microbes by using the entire genome to create a genetic fingerprint. The method involves cutting the whole genome DNA using macro-restrictive (rare cutting) enzymes. Restriction enzymes bind to and cleave specific sites in the genome, and the number of bands each isolate generates is dependent upon the number of restriction sites. The DNA is digested in blocks of agar to prevent physical breakage of the genomic material. The resulting fragments are then separated using electrophoresis, where the direction of the current is repeatedly shifted. This promotes the separation of bands, as larger bands take longer to realign after each switch. The band pattern of the isolate can then be compared to other isolates visually, or by computer software. The more bands the isolates have in common, the closer the relationship (Tenover et al., 1995). Isolates with identical fingerprints (zero band difference) are indistinguishable. If they differ by 2–3 bands, they are considered closely related. If the difference is 4–6 bands, the isolates are possibly related, and if they differ by ≥7 bands, they are considered different (Tenover et al., 1995). PFGE can be used to type a number of bacteria, but the methodology varies between species. A harmonized method has been published for S. aureus (Murchan et al., 2003), but not for S. pseudintermedius. This method has, however, been used, with success, in a plethora of studies regarding S. pseudintermedius (Couto et al., 2016; Davis et al., 2014; Feng et al.,
2012; Gomez-Sanz et al., 2013; Kadlec et al., 2016; Kjellman et al., 2015). Despite the method being old and unable to detect many genetic mutations, PFGE is still a valuable tool in outbreak investigations and assigning genetic relatedness, as it is able to visualize >90% of a genome and is less expensive than whole genome sequencing (WGS) approaches (Goering, 2010; MacCannell, 2013).

2.8.2 Staphylococcal cassette chromosome mec typing

The mecA gene is located in a staphylococcal chromosomal cassette (SCC). SCCs are mobile genetic islands located throughout the staphylococcal genome. These sequences have the ability to capture foreign DNA fragments when the environment becomes hostile to the bacterial cell. SCCmec is a mobile element in which the methicillin resistance gene, as well as other resistance genes, can be transferred between bacteria (IWG-SCC, 2017a). The homology between SCCmec cassettes is considered an indication of horizontal gene transfer between staphylococcal species (Hanssen and Ericson Sollid, 2006).

At the time of writing, there are eleven recognized SCCmec types (IWG-SCC, 2017a). As a common characteristic, the SCCmec elements all carry the mecA or mecC gene, which encode for the altered surface protein PBP2a (IWG-SCC, 2009; Ubukata et al., 1989). SCCmec types are determined by the combination of different ccr (cassette chromosome recombinases) and mec gene complexes (IWG-SCC, 2009). The mec complex contains the mecA gene, as well as mecR1 and mecI, which control the expression of mecA. mecR1 encodes a signal transducer protein (MecR1) and mecI encodes a repressor protein (MecI) (IWG-SCC, 2009). The ccr complex consists of the ccr genes: ccrAB and/or ccrC. These genes enable the SCCmec element to recombine with the staphylococcal chromosome at specific insertion site sequences (ISS). In addition, the SCCmec element also contains three “junkyard regions”, or J regions. These are nonessential sequences contained within the cassette. The length and order of the different components of the cassette vary between different complexes and classes. For example, class A mec gene complexes contain mecA, mecR1, and mecI, as well as IS431 downstream of mecA, while class B mec gene complexes have a truncated mecR1 due to the insertion of IS1272 upstream of mecA. SCCmec elements are also classified into various subtypes depending on the composition of, for example, the J regions. The description of all differences is beyond the scope of this review. The main components of the SCCmec IV are visualized in Figure 2. The ccr and mec gene complexes and their corresponding SCCmec types are presented in Table 1.
SCCmec typing may be used to assess the mobility of mecA, and the identification of the same SCCmec in different strains may indicate the horizontal spread of this genetic element. SCCmec analysis can aid in the further characterization of staphylococci, and in the control and prevention of spread (Liu et al., 2016). A recent study suggested that MRSP isolates carrying SCCmec type II-III could be designated as healthcare associated (HA-MRSP), while MRSP stains with SCCmec type V could be designated as community associated (CA-MRSP) (Kasai et al., 2016). A similar, rough classification has previously been carried out for MRSA, where MRSA strains carrying SCCmec type IV are often considered
community associated, while MRSA strains with type III are often considered healthcare associated (Liu et al., 2016).

2.8.3 Multi-locus sequence typing
Multi-locus sequence typing (MLST) is one of the most valuable methods for long-term and global epidemiology (Bannoehr and Guardabassi, 2012). In this method, specific gene alleles, so-called housekeeping genes, are sequenced and compared to database entries of similar sequences (Maiden et al., 1998). These genes are essential for the metabolism of the microbe and are thus relatively stable to mutation, which results in a slow evolution and change in the genetic sequence (Bannoehr and Guardabassi, 2012). Usually, only an approximately 450 to 500 base pair (bp) fragment is sequenced for each gene, as this is a length for which both DNA strands may be sequenced in their entirety using only one forward and one reverse primer (Enright and Spratt, 1999). Each novel sequence in a certain allele is assigned a number, and the combination of the numbers for each gene allele determines the allelic profile of an isolate, which in turn defines the sequence type (ST) (Enright and Spratt, 1999). STs are further grouped into clonal complexes (CC) based on the similarity of allelic profiles (i.e. how many allele numbers they have in common). Isolates that differ at one locus (SLV, single-locus variant) or two loci (DLV, double-locus variant) are often grouped into the same CC (Damborg et al., 2016; Duim et al., 2016), although official CCs for S. pseudintermedius have yet to be assigned (http://pubmlst.org/spseudintermedius/). A common way of analyzing genetic relatedness from MLST data is eBURST. This method predicts the founding (ancestral) genotype of each clonal complex, and computes the bootstrap support for the assignment (Feil et al., 2004).

The current method for determining the ST of S. pseudintermedius was described by Solyman et al. (2013). In this method, seven different gene loci are sequenced: tuf (elongation factor Tu), cpn60 (chaperonin), pta (phosphotransacetylase), purA (adenylosuccinate synthetase), fdh (formate dehydrogenase), ack (acetate kinase), and sar (sodium sulfate symporter). An earlier method, described by Bannoehr et al. (2007), was based on four of these. At the time of writing this review, well over 700 described STs exist for S. pseudintermedius (http://pubmlst.org/spseudintermedius/).

2.8.4 spa typing
spa typing relies on the analysis of tandem repeat sequences of the staphylococcal protein A (spa) gene, and more specifically, the sequence variation of region X of the gene (Frenay et al., 1996). Moodley et al. (2009) described a method for spa
Review of the literature

typing of MRSP isolates. The method is not suitable for the typing of MSSP isolates, as over 50% of isolates are non-typeable (Bannoehr and Guardabassi, 2012). It is, however, simpler and less time consuming than MLST or PFGE (Bannoehr and Guardabassi, 2012). An online database of identified spa types is available at [http://www.pse-spa.org/spa-types.html](http://www.pse-spa.org/spa-types.html). At the time of writing this review, there are over 70 identified spa types for *S. pseudintermedius*.

2.8.5 Whole genome sequencing

The whole genome sequence of *S. pseudintermedius* was published in 2011 (Ben Zakour et al., 2011). However, while whole genome sequencing (WGS) has been utilized when investigating the epidemiology of MRSA in humans (Azarian et al., 2015; Bartels et al., 2015; Ugolotti et al., 2016), its use has been very limited regarding MRSP. A study by Windahl et al. (2016) did utilize WGS to investigate the relatedness of MRSP isolates from dogs within the same family. Moreover, the whole genome sequences of three predominant STs, 71, 68, and 84, have recently been published (Riley et al., 2016). It is likely that this method, once more affordable, will gradually replace the methods discussed above.

2.9 The epidemiology and occurrence of MRSP

In the 1980s methicillin resistance among *S. (pseud)intermedius* was practically unheard of (Medleau et al., 1986; Phillips and Williams, 1984). The earliest oxacillin-resistant *S. (pseud)intermedius* isolates were discovered from healthy dogs in the mid-1980s, in France (Pellerin et al., 1998). A decade later, MRSP isolates were also being reported in Spain (Piriz et al., 1996), although they were still rare as a whole (Gortel et al., 1999). In 1999, it was determined that MRSP carried the *meca* gene (Gortel et al., 1999). There was, however, an alarming increase in methicillin resistance among *S. (pseud)intermedius* isolates in the United States of America in the early 2000s (Bemis et al., 2009; Jones et al., 2007). Although a French study of clinical isolates from canine pyoderma specimens in 2002 did not detect any methicillin resistance among fifty *S. (pseud)intermedius* isolates (Ganiere et al., 2005), MRSP isolates were detected in Denmark, Switzerland, and Canada in 2004 (Perreten et al., 2010). The next year, the first isolates of multi-resistant, *meca*-positive *S. (pseud)intermedius* were detected in Germany (Loeffler et al., 2007). MRSP was then identified in twelve cases at a veterinary dermatology referral clinic. This was probably the first symptom of the upcoming spread of MRSP ST71 in Europe, which has propagated very rapidly since (Perreten et al., 2010). ST71 is typically resistant to, among others, beta-lactams, gentamicin, chloramphenicol, trimethoprim, macrolides,
lincosamides, tetracycline, and ciprofloxacin (Perreten et al., 2010). It is thus resistant to nearly all antimicrobials authorized for veterinary use in Finland (Lääketietokeskus, 2016). In the following years, MRSP findings were also reported in other European countries: in Sweden, the Netherlands, and Italy (Perreten et al., 2010). As a result of the propagation of MRSP, the proportion of MRSP isolates among *S. pseudintermedius* in the United States, for example, increased from being negligible (<5%) in the 1990’s (Petersen et al., 2002), to >10% in the 2000s (Jones et al., 2007).

Due to differences in methodologies and the origin of isolates, direct comparison of resistance data can be difficult. A Finnish study in the early 2000s examined the susceptibility of certain bacteria in dogs that had or had not received antimicrobial treatment, but did not find any methicillin-resistant staphylococci (Rantala et al., 2004). The first official *meca*-positive MRSP findings in Finland were in 2005–2006, according to surveillance reports published by the Finnish Food Safety Authority Evira (FINRES-Vet, 2007). In all, there were three confirmed MRSP isolates among forty-seven studied. More current data, as well as data from studies from other regions of the world, have been compiled in Table 2.
Table 2  The proportion of MRSP among *Staphylococcus pseudintermedius* isolates or prevalence of MRSP in different studies.

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<td>USA</td>
<td>0</td>
<td>197</td>
<td>Skin lesions from dogs</td>
<td>(Medleau et al., 1986)</td>
</tr>
<tr>
<td>USA</td>
<td>17</td>
<td>336</td>
<td></td>
<td>(Morris et al., 2006)</td>
</tr>
<tr>
<td>USA</td>
<td>16</td>
<td>1317</td>
<td>Year 2005</td>
<td>(Jones et al., 2007)</td>
</tr>
<tr>
<td>USA</td>
<td>30</td>
<td>1755</td>
<td>Year 2007</td>
<td>(Bemis et al., 2009)</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South China</td>
<td>48</td>
<td>144</td>
<td></td>
<td>(Feng et al., 2012)</td>
</tr>
<tr>
<td>Northern China</td>
<td>48</td>
<td>69</td>
<td>Isolates from pyoderma</td>
<td>(Wang et al., 2012)</td>
</tr>
<tr>
<td>South Korea</td>
<td>35</td>
<td>178</td>
<td>SIG, from companion animals and veterinary personnel</td>
<td>(Youn et al., 2011)</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>48</td>
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<td>(De Lucia et al., 2011)</td>
</tr>
<tr>
<td>Poland</td>
<td>12</td>
<td>221</td>
<td>SIG</td>
<td>(Chrobak et al., 2011)</td>
</tr>
<tr>
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<td>8</td>
<td>847</td>
<td>SIG</td>
<td>(Ruscher et al., 2009)</td>
</tr>
<tr>
<td>Portugal</td>
<td>9</td>
<td>446</td>
<td>SIG</td>
<td>(Couto et al., 2016)</td>
</tr>
<tr>
<td>Several</td>
<td>6</td>
<td>605</td>
<td></td>
<td>(Ludwig et al., 2016)</td>
</tr>
<tr>
<td><strong>Nordic countries</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>2</td>
<td>393</td>
<td></td>
<td>(SWEDRES-SVARM, 2016)</td>
</tr>
<tr>
<td>Finland</td>
<td>26</td>
<td>72</td>
<td>Year 2009</td>
<td>(FINRES-Vet, 2011)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>440</td>
<td>Year 2012</td>
<td>(FINRES-Vet, 2015)</td>
</tr>
<tr>
<td>Region</td>
<td>% MRSP</td>
<td>n</td>
<td>Note</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
<td>-----</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td>Brazil</td>
<td>5</td>
<td>148</td>
<td>Skin surface of healthy cats</td>
<td>(Lilenbaum et al., 1998)</td>
</tr>
<tr>
<td>Brazil</td>
<td>4</td>
<td>150</td>
<td>Saliva of healthy cats</td>
<td>(Lilenbaum et al., 1999)</td>
</tr>
<tr>
<td>USA</td>
<td>0</td>
<td>50</td>
<td>Healthy cats</td>
<td>(Abraham et al., 2007)</td>
</tr>
<tr>
<td>USA</td>
<td>0</td>
<td>48</td>
<td>Cats with inflammatory skin disease</td>
<td>(Abraham et al., 2007)</td>
</tr>
<tr>
<td>USA</td>
<td>7</td>
<td>59</td>
<td>Dogs with inflamed skin</td>
<td>(Griffeth et al., 2008)</td>
</tr>
<tr>
<td>USA</td>
<td>2</td>
<td>50</td>
<td>Healthy dogs</td>
<td>(Griffeth et al., 2008)</td>
</tr>
<tr>
<td>Canada</td>
<td>2</td>
<td>193</td>
<td>Dogs entering veterinary hospital</td>
<td>(Hanselman et al., 2008)</td>
</tr>
<tr>
<td>USA</td>
<td>0</td>
<td>200</td>
<td>Healthy cats at an animal hospital</td>
<td>(Gingrich et al., 2011)</td>
</tr>
<tr>
<td>USA</td>
<td>3</td>
<td>200</td>
<td>Healthy dogs at an animal hospital</td>
<td>(Gingrich et al., 2011)</td>
</tr>
<tr>
<td>Canada &amp; USA</td>
<td>4</td>
<td>549</td>
<td>Dogs admitted for TPLO surgery</td>
<td>(Nazarali et al., 2015)</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>53</td>
<td>57</td>
<td>SIG, dogs at a veterinary hospital</td>
<td>(Sasaki et al., 2007a)</td>
</tr>
<tr>
<td>South Korea</td>
<td>26</td>
<td>110</td>
<td>SIG, hospitalized dogs and cats</td>
<td>(Youn et al., 2011)</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slovenia</td>
<td>1,5</td>
<td>200</td>
<td>Clinically healthy dogs</td>
<td>(Vengust et al., 2006)</td>
</tr>
<tr>
<td>Germany</td>
<td>7</td>
<td>814</td>
<td>Dogs at a veterinary hospital</td>
<td>(Nienhoff et al., 2011b)</td>
</tr>
<tr>
<td>Germany</td>
<td>2</td>
<td>131</td>
<td>Cats at a veterinary hospital</td>
<td>(Nienhoff et al., 2011a)</td>
</tr>
<tr>
<td>Norway</td>
<td>3</td>
<td>189</td>
<td>Healthy dogs at animal clinics</td>
<td>(Kjellman et al., 2015)</td>
</tr>
<tr>
<td>Australia</td>
<td>1</td>
<td>117</td>
<td>Healthy dogs in Central Victoria</td>
<td>(Bean and Wigmore, 2016)</td>
</tr>
</tbody>
</table>

*, Percentages have been rounded; n, number of isolates investigated; SIG, results were reported as *Staphylococcus intermedius* group; TPLO, tibial plateau leveling osteotomy.

### 2.9.1 Transmission and carriage of MRSP

Staphylococci typically cause purulent infections, and the bacteria in the pus are easily spread through direct or indirect contact (Caveney et al., 2012; Quinn et al., 2011). Close contact (i.e. animals in the same family) seems to readily spread...
MRSP (Laarhoven et al., 2011; van Duijkeren et al., 2011b). Colonized animals showing no signs of infection may inadvertently spread the bacteria if they are not identified (Laarhoven et al., 2011).

It is uncertain whether animals can eliminate the bacteria and how long a carrier state lasts. One study found that the median length of MRSP carriage was 11 months when the criterion for clearance was two consecutive negative screening specimens (Windahl et al., 2012). However, extended (≥3 weeks) antimicrobial therapy may prolong the carrier state (Windahl et al., 2012). Laarhoven et al. (2011) followed twelve MRSP-positive dogs for six months with monthly sampling, and reported that only two were continuously MRSP positive, while five were intermittently positive and four became negative (the criterion for this was not specified). The last dog was only positive in the initial sample. For MSSP, however, it seems that the bacterium persists in dogs despite attempted decolonization therapy (Saijonmaa-Koulumies et al., 1998).

2.9.2 MRSP in the veterinary environment

MRSP may spread at veterinary hospitals and clinics. This includes spread in the environment, via fomites such as instruments, by veterinary personnel, and by colonized and infected patients (Bergstrom et al., 2012; Lehner et al., 2014; van Duijkeren et al., 2011b; Youn et al., 2011). *S. pseudintermedius* can be isolated from inanimate surfaces at veterinary facilities (Youn et al., 2011). This was also confirmed for MRSP, in a study by Bergström et al. (2012), who isolated MRSP on human and animal contact surfaces in the hospital environment. These included, among others, the entrance and waiting room floor, light switches, door and lamp handles, and taps in the intensive care unit (ICU), isolation ward, and dermatology room. The results indicated that the hospital environment was a potential source of infection in at least two cases. The study also reported a significant increase in the number of dogs that carried MRSP before and after surgery and a stay in the hospital, although the change was small (1/45 to 5/45, \(P<0.001\)).

Spread via contaminated surfaces is probably facilitated by the ability of *S. pseudintermedius* to form biofilms (Bardiau et al., 2013; Han et al., 2015; Osland et al., 2012). A study by Han et al. (2015) found that the vast majority (93.5%) of both MSSP and MRSP isolates were strong biofilm producers. Biofilm production makes bacteria hardy to cleaning and disinfection, and more resistant to antimicrobials (Olsen, 2015), although a clean, dry surface is not a favorable environment for biofilm formation (O'Toole et al., 2000). Antimicrobial resistance due to biofilm formation was specifically demonstrated for *S. pseudintermedius* by Walker et al. (2016). The authors reported that resistance to amikacin, enrofloxacin, cefazolin, and gentamicin was considerably higher.
(nearly or over 1,000-fold) for both MSSP and MRSP when they were allowed to grow in a biofilm. Ferran et al. (2016) also reported high tolerance to antimicrobials for *S. pseudintermedius* in a biofilm.

### 2.9.3 MRSP in households

MRSP is often encountered in the home environment of carrier animals (Laarhoven et al., 2011; van Duijkeren et al., 2011b). It has also been shown that transmission between an MRSP carrier and another pet in the household is common (Laarhoven et al., 2011; van Duijkeren et al., 2011b; Windahl et al., 2016). Laarhoven et al. (2011) found that contact pets were MRSP positive in six out of seven households. In one case, the index dog became MRSP negative during follow-up sampling, while the contact animal remained positive. This is contrary to what Windahl et al. (2016) found, as contact dogs were only positive if the index dog was still positive.

In a larger study involving twenty households, it was discovered that 36% of contact dogs (n = 14) and 31% of contact cats (n = 13) were MRSP positive (van Duijkeren et al., 2011b). The study also found that contamination by MRSP of the household environment was widespread, as 44% of all environmental specimens taken were MRSP positive.

### 2.9.4 *S. pseudintermedius* and MRSP as a zoonosis

Several studies have determined that *S. pseudintermedius* can spread from animals to humans (Gomez-Sanz et al., 2013; Guardabassi et al., 2004; Hanselman et al., 2009; Soedarmanto et al., 2011; van Duijkeren et al., 2008; van Duijkeren et al., 2011b). Human colonization with the bacterium is, however, considered to be a quite rare, although veterinary professionals may carry it at a higher rate than owners (Hanselman et al., 2009; Talan et al., 1989b). One study found that the bacterium could be repeatedly identified from the nasal cavity in dog owners (Gomez-Sanz et al., 2013). However, as humans are not the predominant hosts of *S. pseudintermedius*, it seems that the period of colonization in humans is relatively short (Guardabassi et al., 2004; Paul et al., 2011), although long-term studies are lacking.

*S. (pseud)intermedius* has also been identified as a cause of bite wound infections for nearly two decades (Lee, 1994; Talan et al., 1989a). The bacterium can also cause deep infections in humans, as was reported by van Hoovels et al. (2006), who isolated the bacterium from an infection involving an implantable cardioverter-defibrillator. *S. (pseud)intermedius* has also been reported as a cause of bacteremia (Vandenesch et al., 1995), a brain abscess (Atalay et al., 2005), and pneumonia (Gerstadt et al., 1999) in people. MRSP ST71 has
additionally been reported as a cause of human infections in a small, four-patient cluster at a tertiary care hospital in Sweden (Starlander et al., 2014). While no animal source was identified in these cases, humans with an infection caused by *S. pseudintermedius* commonly have canine contacts (Lozano et al., 2017; Somayaji et al., 2016).

In the case described by van Hoovels et al. (2006), *S. pseudintermedius* had originally been misidentified as *S. aureus*. This is not an isolated case of misidentification, as was shown by Börjesson et al. (2014). Their study concluded that 13% of the *S. aureus* isolates retrieved from dog bite wounds in humans had been misdiagnosed, and were in fact *S. pseudintermedius*. One of these carried the mecA-gene, i.e. was MRSP.

Human cases of MRSP colonization and infection are less reported. Hanselman et al. (2009) found that 0.4% of humans (n = 242) living in the same household as a dog or cat carried MRSP. Veterinary professionals are also exposed to MRSP and are thus at risk of being colonized or infected by it (Guardabassi et al., 2004; Morris et al., 2010; Paul et al., 2011).

In addition to the direct spread of the bacteria, the horizontal spread of resistance genes via the spread of mobile genetic elements (e.g. SCCmec) into human pathogens is also a concern. While there is no direct evidence of SCCmec elements transferring between *S. pseudintermedius* and, for instance, *S. aureus*, studies have shown significant homology between these genes among other staphylococci, i.e. *Staphylococcus epidermidis*, *S. scuiri*, and *S. haemolyticus* (Hanssen and Ericson Sollid, 2006; Hanssen et al., 2005; Hanssen et al., 2004; Wisplinghoff et al., 2003). It is therefore reasonable to consider that the transfer of resistance genes is possible (Guardabassi et al., 2004).

### 2.9.5 Molecular epidemiology of MRSP

The molecular epidemiology of MRSP has recently been reviewed (Pires Dos Santos et al., 2016). An international study by Perreten et al. (2010) investigated the genetic characteristics of MRSP isolates from the United States (California, North Carolina, and Tennesee), Canada (Ontario), as well as several European countries (Denmark, Germany, Italy, the Netherlands, Sweden, and Switzerland). The study found that the MRSP population had spread clonally on both sides of the Atlantic Ocean. ST68 was the most frequent ST among isolates from North America, while ST71 had established itself as the most common clone among European isolates. Since then, ST71 has also been described as a major clone in Japan (Bardiau et al., 2013) and China (Boost et al., 2011; Wang et al., 2012). SCCmec II-III is almost exclusively carried by CC71 isolates (Couto et al., 2014; Ishihara et al., 2016; Perreten et al., 2010). While ST68 and related isolates
(CC68) are still most common in North America, one-third of isolates belonging to this CC originate in Europe (Pires Dos Santos et al., 2016).

In recent years, studies have indicated a change in the population structure of MRSP in several countries. Reports from Sweden (SWEDRES-SVARM, 2015, 2016), Denmark (Damborg et al., 2016), and the Netherlands (Duim et al., 2016) all indicate that ST71 is being displaced by a more heterogeneous MRSP population. However, isolates belonging to CC258 (i.e. ST258 and related clones) have become more common since 2010 (Damborg et al., 2016; Duim et al., 2016; SWEDRES-SVARM, 2016). This CC has been predominantly identified in MRSP clones from Europe (98%) (Pires Dos Santos et al., 2016). Nevertheless, the SWEDRES-SVARM report, as well as the studies by Duim et al. (2016) and Damborg et al. (2016), also describe a plethora of other STs in the MRSP population. While SCCmec IV is widely described among CC258 isolates (Damborg et al., 2016), it has also been described in other, unrelated STs, such as ST268 and 286 (Damborg et al., 2016), and ST106 (Paul et al., 2011). This SCCmec type has even been confirmed to spread between staphylococcal species (Smyth et al., 2011). It has also been indicated that the fitness cost of carrying this SCCmec type is negligible (Lee et al., 2007). Other SCCmec types also appear to be associated with specific CCs. SCCmec type II-III is predominantly found in CC71 isolates (Perreten et al., 2010). This SCCmec type is the result of a combination of SCCmec II (from S. epidermidis) and SCCmec III (from S. aureus) (Descloux et al., 2008). Furthermore, SCCmec type V has been associated with CC68, CC45, and CC379 (Pires Dos Santos et al., 2016).

Another common worldwide clone is CC45 (i.e. ST45 and its related strains e.g. ST83, 113, 179 etc.). Members of this CC have been detected in Europe (Damborg et al., 2016; Duim et al., 2016; SWEDRES-SVARM, 2016), the Middle East and Asia (Perreten et al., 2013), and Australia (Siak et al., 2014). Interestingly, a Norwegian study did not find any CC45 isolates (Kjellman et al., 2015). CC45 isolates are commonly non-typeable by SmaI PFGE and common SCCmec methods. Isolates belonging to this CC generally carry a pseudo-SCCmec element (ΨSCCmec57395), which was described by Perreten et al. (2013).

2.10 Risk factors for MRSP

Several studies have investigated potential risk factors for MRSP. Studies can be roughly divided into those that have investigated risk factors for an animal to be an MRSP carrier (Beck et al., 2012; Hanselman et al., 2009; Nazarali et al., 2015; Nienhoff et al., 2011b; Windahl et al., 2012), and those that have investigated the risk factors for an S. pseudintermedius isolate to be MRSP (Eckholm et al., 2013; Huerta et al., 2011; Kasai et al., 2016; Lehner et al., 2014).
For carriage of MRSP, important risk factors are former hospitalization and antimicrobial therapy (Nienhoff et al., 2011b). It has also been proposed that antimicrobial therapy prolongs the carriage of MRSP (Windahl et al., 2012). A Canadian study by Beck et al. (2012) found that dogs that had previously given negative screening specimens for MRSP had an increased risk of acquiring MRSP if they had been treated with clindamycin. The same study also concluded that MRSP acquisition during treatment of pyoderma seemed to be common (8% pre-treatment vs. 27% post-treatment), and that the prevalence of MRSP among dermatological patients was high (40.5% of skin cultures, 34.1% of screening specimens among 173 dogs). Among dogs and cats, the former appear to carry MRSP at a higher rate, (Hanselman et al., 2009; Nienhoff et al., 2011a; Nienhoff et al., 2011b; Ruscher et al., 2009). Dogs therefore also more commonly have an infection caused by MRSP (Kadlec et al., 2010; Morris et al., 2006). A study by Lehner et al. (2014) that compared the signalment and medical data of 150 MRSP cases to 133 MSSP controls did, however, find indications that being a cat may be a risk factor for MRSP. The number of cats in the control group was nevertheless too small to draw a decisive conclusion.

Antimicrobial therapy has also been indicated as a risk factor for an S. pseudintermedius isolate being MRSP (Eckholm et al., 2013; Huerta et al., 2011; Kasai et al., 2016; Lehner et al., 2014). Kasai et al. (2016) investigated 282 clinical S. pseudintermedius isolates from 462 dogs in Japan. The study revealed that antimicrobial therapy in general, and treatment with beta-lactams (including cepalexin, and third-generation cephalosporins), was a risk factor for the isolate being MRSP (vs. MSSP). Furthermore, they found that treatment with fluoroquinolones, topical treatment with mupirocin, the number of antimicrobials, underlying disease, age, and hospitalization and surgery increased the risk of MRSP. Previous hospitalization or repeated veterinary visits have also been implicated as risk factors for MRSP (vs. MSSP) (Eckholm et al., 2013; Lehner et al., 2014).

Furthermore, other sporadic risk factors have been identified. Huerta et al. (2011), when investigating risk factors associated with resistance in staphylococci in general, reported that animals residing in rural areas were at higher risk of having MRSP. In addition, systemic glucocorticoid therapy was reported as a risk factor by Nienhoff et al. (2011b). This was, however, probably attributable to other associated factors, such as frequent veterinary visits or antimicrobial therapy. The findings of Lehner et al. (2014) were similar, as glucocorticoid therapy was a significant risk factor. A study by Beck et al. (2012) did, however, propose the opposite, as glucocorticoid or cyclosporine therapy tended to decrease the likelihood of MRSP. Concerning surgical site infections (SSI) caused by MRSP, Nazarali et al. (2015) investigated the probability of an SSI caused by MRSP and other bacteria after TPLO surgery among MRSP carriers and non-carriers. Interestingly, the study concluded that bulldogs were at a higher risk of SSI by
either MRSP or any other pathogen within 30 days after TPLO surgery. Furthermore, the study found that postoperative antimicrobial administration was a protective factor against SSIs. The same effect was shown for orthopedic surgeries in general in a study by Pratesi et al. (2015). They noted that dogs receiving postoperative cephalaxin or potentiated amoxicillin developed an SSI in 4% of cases (n ~ 50), while animals that did not receive antimicrobials after surgery developed SSIs in 21% of cases (n = 47). The study did not investigate the probability of developing resistance due to the therapy. However, in human medicine, postoperative use of antimicrobials to prevent SSIs is not recommended by the World Health Organization (WHO), as there is evidence that such measures are not necessary to prevent SSIs (Kang et al., 2009; Khariwala et al., 2016; Lopez and Molina, 2015; WHO, 2016).
3 Aims of the study

The aims of this study were to:

1. Determine the prevalence of MRSP;
2. Investigate the epidemiology of and risk factors for MRSP;
3. Study the molecular epidemiology of MRSP in Finland and how it has changed over time; and
4. Investigate the antimicrobial susceptibility trends of *Staphylococcus pseudintermedius* in Finland.
4 Materials and methods

4.1 Study setting

This study was conducted at the Clinical Microbiology Laboratory (CML) of the Faculty of Veterinary Medicine, University of Helsinki. The CML provides bacteriological analysis services to the University of Helsinki Veterinary Teaching Hospital (VTH) and private veterinary clinics and veterinarians throughout Finland. The study utilized data and isolates of the CML database and strain collection, as well as specimen information stored in the laboratory information system and the VTH patient information system. In 2011–2015, the laboratory processed 19,249 microbiological specimens, of which 6889 (32%) were pus specimens, 5990 (28%) were urine specimens, 3573 (17%) were from miscellaneous sources (respiratory, fecal etc.) and 514 (2%) were blood cultures. The rest, 4296 (20%), were specimens for screening of resistant bacteria (MRSP, MRSA or ESBL), predominantly collected from animals with risk factors. The majority (67%) of the specimens were from the VTH, while the rest were from private veterinary clinics. Apart from the investigation of clinical specimens, the laboratory is responsible for resistance surveillance of small animal pathogens in the hospital and in Finland.

For study I, the risk factors for MRSP during an MRSP outbreak at the VTH were studied among hospitalized patients of the VTH. The VTH is a national primary care and referral animal hospital in Finland. The hospital provides 24/7 emergency and intensive care services primarily for animals in the Greater Helsinki area. The Small Animal Hospital of the unit has approximately 18,000 visits annually, with nearly 2000 surgical procedures. Approximately 80% of patients are dogs, 17% cats, and the rest are other species.

In study II, the prevalence of MRSP and MRSA, as well as risk factors for these, were studied amongst the guide dog population of the Guide Dog School (GDS) of the Finnish Federation of the Visually Impaired. The school is a nonprofit organization whose mission is to enhance the independence of people who are blind or visually impaired through the use of specially trained dogs, and is situated in the greater Helsinki area in Finland (http://opaskoirakoulu.fi/?lang=en). The school breeds the majority of their dogs themselves, and breeding bitches are housed in volunteer families. The bitches give birth at the GDS facilities in a separate ward reserved for this purpose. Puppies spend their first six weeks with their dam at the school, after which they are weaned and housed in volunteer families. During the first year of their life, the dogs have regular (short) appointments at the school. At the age of 13–18
months, guide dog candidates are transferred back to the school, where they undergo suitability testing. If selected, the dog begins a training period of 20 weeks, after which it is placed for service as a guide dog. During their service, the dogs spend short periods at the school in training; the GDS facilities also serve as a kennel for guide dogs during vacations of their host or hostess.

This study was undertaken because in 2012–2013, MRSP was discovered in routine clinical specimens from three GDS dogs with atopic dermatitis. The dogs of the GDS constitute a special group of animals; they are in close contact during training at the GDS kennel, after which they are sent all over the country. During their service years, they also regularly visit the school’s premises. Thus, the nationwide risk for the spread of resistant bacteria is a valid concern. At the time the study was planned, the size of the guide dog population was approximately 330 dogs. Out of these, roughly 110 dogs were in training, 200 worked as guide dogs, and 20 animals were breeding bitches.

For study III, bacterial isolates for susceptibility data originated from clinical and screening specimens that had arrived to the CML in 2011–2015. To investigate the molecular epidemiology of MRSP, all MRSP isolates stored at the CML in 2010–2014 were used.

4.2 Specimen collection (I–III)

Clinical specimens (i.e. specimens from infection sites, such as wounds, abscesses, and peritoneal fluid) were collected by the treating veterinarian. Specimens from open lesions were largely retrieved by swabbing the lesions with a sterile swab with transport medium (M40 Transsystem Amies Agar Gel, with or without charcoal, Copan Diagnostics Inc., Italy). Specimens from deep closed lesions (synovial fluid, abscesses, etc.) were mainly collected into a transport vial (Port-F, Biomerieux, France).

Screening specimens from dogs and cats were collected by swabbing (as above) the mucous membranes of the nose and mouth of the animal, as well as the perineum. If the animal had any skin lesions, these were also swabbed. Patients screened for MRSP commonly have identified risk factors for MRSP, such as frequent antimicrobial exposure, chronic or intermittent infection, such as pyoderma, surgical site infection, or previous exposure to MRSP (either in hospital or family).

Clinical specimens may have revealed any bacteria that were capable of growth on standard media (see section 4.3), while screening specimens could only reveal MRSP or MRSA.

Screening specimens for study II were collected between February and November 2014, when the dogs visited the GDS. This method was selected out of convenience instead of random sampling. Additionally, dogs in the same family
of MRSP-positive guide dogs were also screened for MRSP. The specimens in this study were then transferred to the CML, where they were kept refrigerated at +4°C if they arrived outside office hours. All specimens were processed within two days of sampling.

4.3 Bacteriological culture (I–III)

Specimens were cultured according to the standard methods of the laboratory. In short, specimens from superficial lesions were cultured onto a blood agar plate (Tryptone Soy Agar with 5% Sheep Blood, Oxoid, Germany), as well as an assortment of different selective and non-selective agars, depending on the specified specimen site. Specimens from deep open and closed lesions were additionally cultured anaerobically using at least a fastidious anaerobe agar with 5% horse blood (Oxoid, Germany). These specimens were also placed in an enrichment broth (Fastidious Anaerobe Broth, Tammer-Tutkan Maljat Oy, Finland) to increase the sensitivity to detect bacterial growth.

Screening specimens were cultured by pooling all swabs taken from one animal (2–3 swabs) into a tryptic soy broth supplemented with 6.5% NaCl (Tammer-Tutkan Maljat, Finland). After overnight incubation, an aliquot was plated onto an MRSA-selective agar (MRSA Select, BioRad, USA). Plates were inspected for typical growth (MRSA: red-pink colonies; MRSP: light-pink colonies) after 24 and 48 hours. If typical growth was observed, colonies were pure cultured onto blood agar plates.

4.4 Species identification (I–III)

Presumptive identification of *S. pseudintermedius* was based on the typical colony morphology and hemolysis pattern (double hemolysis, with complete hemolysis [α-toxin] below and near the colony and incomplete hemolysis [β-toxin] further out), a positive tube rabbit plasma coagulation test (BBL Coagulase Plasma, Becton Dickinson, USA), and susceptibility to polymyxin B (300 U, Oxoid Ltd, UK) (sensitive ≥10 mm, resistant <10 mm), acid formation from trehalose and sucrose (Diatabs, Rosco Diagnostica A/S, Denmark), and the source animal’s species (canine and feline isolates more likely to be *S. pseudintermedius*) (Devriese *et al.*, 2009; Devriese *et al.*, 2005). If in doubt, testing was expanded to maltose and xylose sugars and/or Staph ID32 (bioMérieux SA, France). If an isolate did not meet all these criteria, it was characterized as SIG and was omitted from the study. Molecular typing methods for MRSP isolates were also utilized to definitively confirm the species.
4.5 Susceptibility testing (I–III)

Susceptibility testing for all studies was performed using the disk diffusion method (Oxoid Ltd., UK) in accordance with the Clinical and Laboratory Standards Institute (CLSI) performance standards (CLSI, 2013a, b). All breakpoints were derived from this standard, except for the breakpoint for fusidic acid, which was derived from the FiRe standard (version 6) (National Institute of Health and Welfare, 2009). Furthermore, a nitrocefin disc test was used (BBL Cefinase Paper Disc, Becton, Dickinson & co., USA) to determine whether an isolate produced beta-lactamase. The disk was applied to the zone edge surrounding the oxacillin disk on Müller-Hinton agar (CLSI, 2013a, b). The type strain *Staphylococcus aureus* ATCC® 25923™ was used as a control for susceptibility testing.

4.6 Study populations and data collection

4.6.1 Study I

The purpose of this study was to describe the MRSP hospital outbreak at the VTH and determine the risk factors for acquiring MRSP during the outbreak. The study population consisted of dogs and cats that had been hospitalized for 1 day or more at the VTH during the outbreak period (November 2010 to January 2012), and were thus potentially exposed to nosocomial MRSP. Cases were divided into either colonized (MRSP cultured only from mucous membranes) or infected (MRSP cultured from an infection site) with MRSP displaying the outbreak antibiogram; resistance to oxacillin, erythromycin, clindamycin, sulfamethoxazole-trimethoprim, gentamicin, tetracycline, and enrofloxacin, and susceptibility to fusidic acid and amikacin. Only infections detected during the outbreak period, either after surgical procedures performed at the hospital or other infections that appeared after prolonged or several treatment periods in the hospital, were included, in order to exclude community-acquired MRSPs. Colonized patients were enrolled if the MRSP was detected after at least 1 day of hospitalization and the animal had been treated in the same wards as MRSP-positive patients. Controls were patients from the same population as cases, but were negative in MRSP screening. Patients with a positive MRSP specimen on first admission and non-hospitalized (policlinic) patients were excluded from the study. Data on several potential risk factor variables were collected for all included patients (Table 3).
Table 3  
Risk factor variables analyzed from cases and controls during the MRSP outbreak at the Veterinary Teaching Hospital of Helsinki University in 2010–2011.

<table>
<thead>
<tr>
<th>Species (dog/cat)</th>
<th>Emergency surgery (during weekend/evening/night)</th>
<th>Aminopenicillin medication given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Length of anesthesia (min)</td>
<td>Days of aminopenicillin therapy</td>
</tr>
<tr>
<td>Gender</td>
<td>Days in hospital</td>
<td>Cephalosporin medication given</td>
</tr>
<tr>
<td>Breed</td>
<td>Days in surgery ward</td>
<td>Days of cephalosporin therapy</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Days in intensive care unit</td>
<td>Enrofloxacin medication given</td>
</tr>
<tr>
<td>Severity of condition</td>
<td>Days in other wards</td>
<td>Days of enrofloxacin therapy</td>
</tr>
<tr>
<td>Skin lesions of any cause</td>
<td>Antimicrobial medication given</td>
<td>Proton pump inhibitor (PPI) given</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td>Days of any antimicrobial therapy</td>
<td>Days of PPI therapy</td>
</tr>
</tbody>
</table>

a Severity was assessed by the author (TG) on a scale of 1 to 5 after reviewing the patient record on admission and was based on the guidelines provided by the American Society of Anesthesiologists.

b The same patient might have had several visits or courses of medication. Therefore, the cumulative number of days for these variables was recorded until the first positive MRSP specimen (cases), or latest negative MRSP specimen (controls); see text for details.

c Enrofloxacin was the only fluoroquinolone used for these patients.

4.6.2 Study II  
This study was undertaken to determine the prevalence of MRSP and MRSA at the GDS and identify risk factors for an animal carrying MRSP. The sample population consisted of guide dogs that visited the GDS (a convenience sample), while the target population was all dogs of the GDS. To identify possible risk factors for MRSP, the following data were collected from the dogs of the sample population: breed, sex, age, whether the animal was bred by the GDS or purchased, whether it was a trainee, at service or a breeding bitch, and medical history. Medical history included information on previous skin disease, and antimicrobial and other medical treatments within a 12-month period prior to entering the study. The age, sex, breed, and dog group variables, as well as the exact number of dogs in the whole population, and different subgroups were collected from the entire target population, in order to assess the representativeness of the sample population.

4.6.3 Study III  
For the analysis of antimicrobial susceptibility and yearly trends in antimicrobial resistance, results for all clinical isolates identified as S. pseudintermedius between June 2011 and the end of 2015 at the CML were compiled from the
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laboratory information system (LIS) (Provet Net, Finnish Net Solutions, Finland). This time period was selected to ensure consistent data, as an overhaul of the LIS was carried out in May 2011. Isolates originated from both the VTH as well as private veterinary clinics and were identified from either clinical specimens or screening specimens. In addition, patient information data were collected on the species, sex, specimen type, presence of antimicrobial therapy at the time of sampling, and submitting clinic. Antimicrobials tested in the basic panel included clindamycin, erythromycin, fucidic acid, oxacillin, sulfamethoxazole/trimetoprim and tetracycline. Comparable data for the extended panel (amikacin, chloramphenicol, doxycycline, enrofloxacin, and gentamicin) were only available for 2015. Denominator data on the numbers of specimens per time period were also extracted from the LIS. MRSP isolates stored in 2010–2014 were used for molecular analysis.

4.6.4 Unpublished data
To determine the incidence and distribution of MRSP in Finland, specimen and denominator data from study III were used to calculate the incidence of MRSP in clinical specimens. To determine the distribution of MRSP in Finland, the geographic origin of a specimen in study III was assigned according to the postal address zip codes of the submitting clinic. These were then grouped according to the jurisdictional regions of the six Regional State Administrative Agencies (referred to only as regions) of continental Finland.

Furthermore, oxacillin susceptibility histograms and the production of beta-lactamase among S. pseudintermedius isolates were investigated. Data on susceptibility histograms for oxacillin in 2011 and 2015 were collected from WHONET using the same data as in study III. For the production of beta-lactamase, consistent laboratory data were only available for the year 2016.

4.7 Data analysis and statistical methods
All statistical analyses for studies I and II were performed using the SAS System for Windows, version 9.3 (SAS Institute Inc., USA). For study III, statistical analyses were performed with SAS (as above) to compare the differences in resistance between MRSP and MSSP, as well as time-trend analyses, and using SPSS version 24 (IBM Inc., USA) for risk factor analyses. $P$-values $<0.05$ were considered statistically significant in all studies.
4.7.1 Study I
Descriptive analysis of cases related to the outbreak was performed by presenting the number of new cases per week over the outbreak and follow-up periods in the epidemic curve, along with the implemented control measures. The number of colonized and infected patients was recorded. The attack rate was determined by using the number of hospitalized patients as the denominator. The risk factors for acquiring MRSP were assessed by logistic regression among 55 cases and 213 controls. Each factor was first modeled using a univariable logistic regression model. To control for confounders, a stepwise multivariable logistic regression analysis was conducted for the risk factors with a $P$-value $\leq 0.05$ in the univariate analyses. In the stepwise selection process, a significance level of 0.15 was required to allow a variable into the multivariable model, and a significance level of 0.20 was required for a variable to remain in the multivariable model. Odds ratios (OR) with 95% confidence intervals (CI) were calculated.

4.7.2 Study II
The sample size for the entire 330-dog population was calculated using EpiTools (AusVet-a). The estimated sample size was 152 dogs with an approximated MRSP prevalence of 3%, with a $\pm 2\%$ desired precision at a 95% confidence level. The guide dogs were considered a low-risk population, and the three percent estimate was thus based on previous studies that were considered to represent our target population. These showed a prevalence between 1.5 and 4.5 percent (Gingrich et al., 2011; Griffeth et al., 2008; Hanselman et al., 2008; Hanselman et al., 2009; Vengust et al., 2006). It was not considered necessary to calculate the sample size for MRSA separately, since the prevalence of MRSA was expected to be less than that of MRSP.

The prevalence estimates for MRSP and MRSA were calculated as the number of positive specimens divided by the total number of collected specimens and presented as percentages. The 95% confidence intervals (95% CI) for the prevalence estimates were determined using an EpiTools calculator (AusVet-b). Confidence intervals were reported based on the Wilson score interval due to the low prevalence (Brown et al., 2001). The representativeness of the sample population was assessed by using the Fisher’s exact test for sex, breed, and dog group, and using an independent two-sample $t$-test for age.

Descriptive statistics for the studied variables were calculated according to the MRSP status using univariable logistic regression models. Due to very few positive MRSP results in the data, the rareness of the events was taken into account in the modeling by applying Firth’s bias adjustment method (Firth, 1993), which maximizes a penalized likelihood function, instead of the standard maximum likelihood function. Odds ratios (OR) with 95% profile likelihood
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(Venzon and Moolgavkar, 1988) confidence intervals (CI) were calculated to quantify the results.

4.7.3 Study III
Susceptibility data for clinical S. pseudintermedius isolates (screening specimens excluded) were analyzed using WHONET (v. 5.6, WHO). Non-susceptibility percentages, including resistant and intermediate isolates, with 95% confidence intervals (CI95%) were separately calculated and presented for MRSP, MSSP (methicillin-susceptible S. pseudintermedius) and all S. pseudintermedius isolates. CLSI breakpoints were used (CLSI, 2013a, b), except for fusidic acid, for which a non-susceptibility breakpoint of ≤23 mm was used (National Institute of Health and Welfare, 2009). Yearly trends for non-susceptibility percentages were plotted and trends were investigated using a Cochran-Armitage trend test for each antimicrobial. The statistical difference in non-susceptibilities between MRSP and MSSP was investigated using Pearson’s chi-squared test based on the WHONET output. To calculate the number of MDR isolates (resistance to ≥3 antimicrobial classes), macrolide and lincosamide resistance was pooled due to common MLSB resistance (macrolide, lincosamide, and streptogramin B) (Giguère, 2013a).

The proportions of MRSP in clinical and screening specimens were calculated for dogs and cats in order to derive crude prevalence estimates for MRSP in dogs and cats seeking veterinary care, from which microbiological specimens are obtained, and in high-risk populations, respectively.

For the analysis of predictors of MRSP, data were separately analyzed for clinical S. pseudintermedius isolates and screening specimens by logistic regression with MRSP as the outcome variable. As data from the MRSP outbreak at the VTH in 2010–2011 were likely to skew the results, data from this period were omitted. Due to the low number of cats in the data (n = 18 for clinical specimens and zero positive out of 145 for screening specimens), these, as well as specimens from unknown species (n = 11), were omitted from the analyses. OR with CI95% and P-values were calculated for each variable. Variables with a P-value ≤0.2 in the univariable analysis were included in the multivariable analysis. Multivariable logistic regression was performed using a backward step (Wald) method.

To compare the genetic relatedness MRSP isolates, the allele sequences for each ST were added back-to-back (ack, cpn60, fih, pta, purA, sar, tuf). The resulting sequences were aligned and a phylogenetic tree was inferred by the Bayesian Markov chain Monte Carlo method implemented in BEAST (v. 1.7.2) (Drummond and Rambaut, 2007). Each run was continued until the effective sample size was over 200. Posterior probabilities were calculated with a 10%
burn-in, and values >0.7 were considered significant. Results were visualized in FigTree (v. 1.40). Additionally, goeBURST (v 1.2.1, http://www.phyloviz.net/goeburst/) software was used for population structure analysis of STs (Francisco et al., 2012). Analysis was conducted at double- and triple-locus variant levels. Single- and double-locus variants of previously described CCs were assigned to that CC (Duim et al., 2016). The number of isolates per ST or CC per year was calculated based on the specimen collection date.

4.7.4 Unpublished data
The yearly incidence of MRSP among 1000 clinical specimens (regardless of whether they revealed S. pseudintermedius) was calculated as a total value, and for canine and feline specimens separately. The results were plotted in a graph.

To assess any change in the distribution of susceptibility, the distribution of the zones of inhibition (ZOI) for oxacillin in 2011 and 2015 was visualized as a histogram using WHONET. To assess the proportion of MSSP isolates that produce beta-lactamase, laboratory output data were placed in Excel (v. 15, Microsoft Corporation, USA) and the proportion of beta-lactamase-producing MSSP isolates was calculated. The confidence interval for the proportion was calculated using EpiTools (AusVet-c, 2017).

4.8 Molecular methods

4.8.1 Extraction of genomic DNA
Bacterial DNA for mecA PCR, SCCmec, and MLST typing was extracted by boiling (Alexopoulou et al., 2006) in study I, and with a commercial kit (InstaGene Matrix, Bio-Rad, USA) as described previously (DTU Food - National Food Institute, 2012) in studies II and III.

4.8.2 mecA PCR (I–III)
For study I, MRSP isolates were sent to the Finnish Food Safety Authority (Evira) for verification of the presence of mecA (Murakami et al., 1991). For studies II and III, the presence of the mecA gene was confirmed using PCR primers (mecA P4 and mecA P7, see Table 4) described by Stegger et al. (2012). Both primers had a concentration of 0.25 μM in a final reaction volume of 20 μl. The PCR was performed with a BioRad CFX96 Real-Time PCR detection system in a BioRad C1000 Touch thermal cycler using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA). The protocol included an initial denaturation at 98.0 °C for 2
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min, followed by 40 cycles of denaturation (98.0°C for 5 s) and annealing/elongation (60.0°C for 45 s). Finally, a denaturation step (98.0 °C for 5 s) preceded the melt-curve analysis (65.0 °C to 95.0 °C in 0.5 °C increments), which was used to verify the product. The product had a melting point of 77.5–78.0 °C. *Staphylococcus aureus* ATCC® 43300™ was used as a positive control for *mecA* testing.

4.8.3 SCCmec typing (I–III)
The SCCmec cassettes were typed using a previously described multiplex PCR method (Kondo et al., 2007) in study I, while the following modifications were made for studies II and III: in M-PCR-1, primers γR and γF had a final concentration of 0.3 μM, while all other primers in M-PCR-1 and M-PCR-2 had a final concentration of 0.2 μM. Primers are presented in Table 4. The PCRs were performed with Phire Green HotStart II DNA polymerase (Thermo Scientific, USA) with 200 μM of each dNTP in a reaction, or with Phire Green Hot Start II PCR Master Mix (Thermo Scientific, USA). The conditions for the PCRs were as follows: initial denaturation at 98.0 °C for 1 min, followed by 40 cycles of denaturation (98.0 °C for 15 s), annealing (56.0 °C for 10 s) and elongation (72.0 °C for 45 s), and a final elongation for 2 min at 72.0 °C. Bands were visualized using SYBR Safe DNA stain (Life Technologies, USA) after electrophoresis in 1% agarose. The following strains were used for positive controls in SCCmec testing: *S. aureus* NCTC 10442 (I), *S. aureus* ATCC® BAA-1720™ (II), *S. aureus* ATCC® BAA-43™ (III), *S. aureus* JCSC 6944 (V), and *S. aureus* ATCC® BAA-42™ (VI).

4.8.4 Multi-locus sequence typing (I–III)
Multi-locus sequence typing was performed as described by Solyman et al. (2013) for study I. For studies II and III, some modifications were made: Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific, USA) was used for the reaction. Furthermore, the tuf primers had a final concentration of 0.375 μM each, while all other primers had a concentration of 0.25 μM. The PCR protocol consisted of a 15-s initial denaturation at 98 °C, 30 cycles of denaturation (98 °C for 2 s), annealing (52 °C for 10 s), and elongation (72 °C for 15 s), with final extension for 1 min at 72 °C. PCR products were purified using Exonuclease I (Thermo Scientific, USA) and FastAP thermosensitive alkaline phosphatase (Thermo Scientific, USA) according to the manufacturer’s instructions. The sequencing for MLST was performed by a commercial laboratory (Macrogen Inc., Netherlands) with an ABI 3730 XL automated sequencer. The sequences were analyzed using the CLC Main Workbench (version 6.9.1, CLC bio, Denmark) with
the CLC MLST module (version 1.4.7, CLC bio, Denmark) comparing sequences of the housekeeping genes to the *S. pseudintermedius* MLST database ([http://pubmlst.org/spseudintermedius/](http://pubmlst.org/spseudintermedius/)). For many isolates, the amplification of the *tuf* gene was weak when using primers *tuf* forward and *tuf* reverse, as described by Bannoehr *et al.* (2007). To try to improve the results, these primers were elongated by three bp at the 5′ end by choosing corresponding nucleotides as found in the complete genome sequence of *Staphylococcus pseudintermedius HKU10-03* (NCBI GenBank accession no. CP002439) (Tse *et al.*, 2011). The newly designed primers were *tuf*19F (5′-GTCCAATGCCAAACTCG-3′) and *tuf*19R (5′-CCAGCTTCAGCGTAGTCTA-3′). These were used both in the PCR amplification step and as sequencing primers. All used primers are presented in Table 4.
Table 4  Primers used for mecA, SCCmec, and MLST analyses of MRSP isolates identified at the Clinical Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Helsinki, in 2010–2014.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer</th>
<th>Product size (bp)</th>
<th>Sequence (5'→3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA detection</td>
<td>mecA</td>
<td>162</td>
<td>TCCAGATTACAACCTCCACCAGG</td>
<td>(Stegger et al., 2012)</td>
</tr>
<tr>
<td>mecA</td>
<td>mecA</td>
<td>-</td>
<td>CCCTTCATATCCTGTAACG</td>
<td>*</td>
</tr>
<tr>
<td>SCCmec typing (ccr gene complex)</td>
<td>mecA</td>
<td>mA1</td>
<td>TGCTATCCACCTCCACCAAGG</td>
<td>(Kondo et al., 2007)</td>
</tr>
<tr>
<td>mecA</td>
<td>mA2</td>
<td>695</td>
<td>AACCTATACATCATACTGACGTACGT</td>
<td>*</td>
</tr>
<tr>
<td>ccrA1</td>
<td>a1</td>
<td>286</td>
<td>CATAACTTCCATTCTGCAGATG</td>
<td>(Kondo et al., 2007)</td>
</tr>
<tr>
<td>ccrA2</td>
<td>a2</td>
<td>937</td>
<td>TAAAGGCTACATGCAAAACACT</td>
<td>*</td>
</tr>
<tr>
<td>ccrA3</td>
<td>a3</td>
<td>1,791</td>
<td>AGCTCAAAAGCAAGCAATAGAAT</td>
<td>*</td>
</tr>
<tr>
<td>ccrB1-B3</td>
<td>βc</td>
<td>-</td>
<td>ATGGCTTTGATAATAGCCITCT</td>
<td>*</td>
</tr>
<tr>
<td>ccrA4</td>
<td>a4.2</td>
<td>1,287</td>
<td>GTATCAATGCAACAGAATT</td>
<td>*</td>
</tr>
<tr>
<td>ccrB4</td>
<td>β4.2</td>
<td>-</td>
<td>TTGCGACTCTCTTGCGCTTT</td>
<td>*</td>
</tr>
<tr>
<td>ccrC</td>
<td>γR</td>
<td>518</td>
<td>CTTTATAGACTGAGATTACAAAT</td>
<td>*</td>
</tr>
<tr>
<td>SCCmec typing (mec gene complex)</td>
<td>mecI</td>
<td>ml6</td>
<td>CATAACTTCCATTTCTGCAGATG</td>
<td>(Kondo et al., 2007)</td>
</tr>
<tr>
<td>IS1272</td>
<td>IS7</td>
<td>2,827</td>
<td>ATGCTTAATGATAGCATCAGAAGT</td>
<td>*</td>
</tr>
<tr>
<td>IS431</td>
<td>IS2(iS-2)</td>
<td>804</td>
<td>TGAGGTTATGCAATTTGCGATGT</td>
<td>*</td>
</tr>
<tr>
<td>mecA</td>
<td>mA7</td>
<td>-</td>
<td>ATATACCAAACCGACAACCTACA</td>
<td>*</td>
</tr>
<tr>
<td>Multi-locus sequence typing</td>
<td>tuf</td>
<td>F</td>
<td>GTCAGATTGCAACAAACTCG</td>
<td>(Bannoehr et al., 2007)*</td>
</tr>
<tr>
<td>cpn60</td>
<td>cpn60</td>
<td>F</td>
<td>GCGACTGTAATGCAACAGCA</td>
<td>(Bannoehr et al., 2007)</td>
</tr>
<tr>
<td>pta</td>
<td>pta</td>
<td>F</td>
<td>GTGGGTATGATATTACAGAAGG</td>
<td>*</td>
</tr>
<tr>
<td>pta</td>
<td>pta</td>
<td>R</td>
<td>GCAGAACTTTGTTGAGAAGC</td>
<td>*</td>
</tr>
<tr>
<td>purA</td>
<td>purA</td>
<td>F</td>
<td>GATTACTTCCAAGGATGTTT</td>
<td>(Solyman et al., 2013)</td>
</tr>
<tr>
<td>fdh</td>
<td>fdh</td>
<td>F</td>
<td>TGGCATAACAGGATGCGTT</td>
<td>*</td>
</tr>
<tr>
<td>ack</td>
<td>ack</td>
<td>F</td>
<td>CACCACCTCAACACCCAGCAACACT</td>
<td>*</td>
</tr>
<tr>
<td>sar</td>
<td>sar</td>
<td>F</td>
<td>GGTATTAGTCCATGTCACAAAT</td>
<td>*</td>
</tr>
<tr>
<td>bp, base pair; *, Modified for studies II and III by adding the underlined nucleotides, see text; -, complementary primer.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.8.5 Pulsed-field gel electrophoresis (I–III)

A modified version of the HARMONY protocol, as described by Murchan et al. (2003), was used for pulsed-field typing of MRSP isolates. Approximately $4 \times 10^8$ colony forming units per strain plug were suspended in EC buffer (1 M sodium chloride, 0.5% polyoxyethylene 20 cetyl ether, 0.2% w/v sodium deoxycholate, 0.5% w/v N-lauroyl-sarcosine sodium salt, 0.1 M EDTA, 6 mM 1.0 M Tris-HCl). The plugs were made by mixing the bacteria and EC buffer suspension with 20 µl of lysostaphin (1 mg/ml, Sigma-Aldrich, USA), and 200 µl of 2% SeaPlaque GTG agarose (Lonza Inc., USA). The digestion was performed using 10% NEBuffer 4 or CutSmart buffer and 1.5 U SmaI per isolate (New England BioLabs Inc., USA) for 4–18 hours. Isolates that were not typeable using SmaI were macrorestricted using 1.5 U of AscI restriction enzyme per isolate (New England BioLabs Inc., USA). The pulsed-field electrophoresis was carried out in 1% SeaKem agar (Lonza Inc., USA) on the CHEF-DR III system (Bio-Rad Laboratories, USA). The total run time was 22 h; the first-block switch time was 0.1–15 s for 15 h, and the second-block switch time was 15–60 s for 7 h. The voltage for the run was 6 V/cm, with an included angle of 120°.

Gels were stained with SYBR Safe DNA gel stain (Life technologies, USA) and analyzed using GelCompar II v. 6.6 software (Applied Maths NV, Belgium). Cluster analysis was performed by UPGMA (Unweighted Pair Group Method with Arithmetic Mean), based on the Dice similarity coefficient, with optimization and position tolerance both set at 1% for studies I and II. In study III, optimization and position tolerance were both set at 1.3%. Isolates were clustered using an 85% similarity cut-off for studies I and II, and using an 80% cut-off for study III.

4.9 Ethical aspects

Taking screening specimens for MRSP can cause very mild discomfort in the animal. Taking specimens from infection sites is medically necessary. In article I, the information was gathered as part of the infection control strategy of the VTH and was conducted to ensure patient safety in accordance with the hospital’s ethical guidelines. The owners of the animals were informed about the outbreak and study. They agreed to the investigation and the taking of necessary specimens, as well as any attempts to control the outbreak.

In study II, the Guide Dog School of the Finnish Federation of the Visually Impaired gave full consent and actively participated in providing specimens and data. The Viikki Campus Research Ethics Committee has stated (statement 4/2014) that the storage and use of excess animal patient specimens is ethically acceptable, which is also applicable to a study utilizing laboratory data and bacterial isolates, as was done in study III. All patient information in each study was handled confidentially and encrypted by assigning numbers to patients.
5 Results and Discussion

5.1 Hospital outbreak investigation (I)

The MRSP outbreak spanned over a period of 14 months, during which 63 patients were found to be infected \( (n = 27) \) or colonized \( (n = 36) \). The different types of infections caused by MRSP during the outbreak are summarized in Table 5. Fifty-eight of the cases (92%) were dogs and five (8%) were cats. Breed variation was vast among dogs (over 40 different breeds), and all five cats were domestic short-haired cats.

Table 5  
Nosocomial infections \( (n = 27) \) caused by the MRSP outbreak strain \( (ST71, SCCmec II-III) \) in the Veterinary Teaching Hospital of Helsinki University in 2010 – 2011 (Study I).

<table>
<thead>
<tr>
<th>Infection type</th>
<th>Number of infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical site infections (total)</td>
<td>19</td>
</tr>
<tr>
<td>Required surgical revision</td>
<td>3</td>
</tr>
<tr>
<td>Involved orthopedic devices*</td>
<td>7</td>
</tr>
<tr>
<td>Others (uncomplicated)</td>
<td>9</td>
</tr>
<tr>
<td>Other wound infections</td>
<td>3</td>
</tr>
<tr>
<td>Otitis(^b)</td>
<td>1</td>
</tr>
<tr>
<td>Bite wound(^c)</td>
<td>2</td>
</tr>
<tr>
<td>Dermatitis(^d)</td>
<td>1</td>
</tr>
<tr>
<td>Cystitis complicated by uroliths(^e)</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\), some cases required the removal of surgical devices and revision; \(^b\), patient had orthopedic surgery and several visits to the hospital, and otitis was subsequently diagnosed; \(^c\), Both patients presented with severe bite wounds; after a prolonged hospital stay MRSP was cultured from the wound; \(^d\), The patient presented with pneumonia, autoimmune myositis, and dermal vasculitis, and later developed an MRSP infection on the skin lesion; \(^e\), Colonization with MRSP preceded the cystitis.

The index patient was a 3-year-old dachshund that was referred to the VTH emergency care unit at the end of October 2010. The critically ill dog suffered from systemic inflammatory response syndrome and disseminated intravascular coagulation due to necrotizing mastitis. These may have been postoperative complications after a cesarean section and an ovariohysterectomy performed at two different private veterinary clinics. Surgery was performed at the VTH to remove necrotized tissue. The dog was transferred to the intensive care unit.
(ICU), where it was treated for 1 week. A bacteriological culture from the initial infection revealed a pure growth of *Escherichia coli*. Only two days after discharge, a surgical site infection was noted. The bacteriological culture revealed multidrug-resistant MRSP. An outbreak investigation was initiated due to this uncommon finding. This included culturing all infection sites and screening of patients potentially exposed to MRSP.

The overall attack rate of MRSP was 2.1% (63/2969) among hospitalized patients and 3.8% (43/1121) among patients discharged from the ICU. MRSP was the cause of a surgical wound infection in 0.9% of surgical procedures (17/1864). The epidemic curve indicating the number of new cases per week is presented in Figure 3. During the MRSP outbreak at the VTH, the monthly incidence varied between zero and ten per a thousand patients. Peaks in incidence can be seen November 2010 and October 2011 (Figure 4).
Figure 3  An epidemic curve showing new MRSP ST71 cases during the outbreak in 2010–2012 at the Veterinary Teaching Hospital of Helsinki University. The outbreak period was between November 2010 and January 2012, after which the follow-up period was started. A, hospital closed for 2 days for cleaning and disinfection; B, establishment of cohort ward; C, nurse responsible for hospital hygiene appointed; D, hospital closed for 5 days for cleaning and disinfection; E, veterinarian appointed as infection control officer; S, screening of hospitalized patients; H, environmental swabs taken (Study I).
The monthly cumulative incidence of all MRSPs and MRSPs displaying the outbreak
antibiogram (MRSP ST71) among patients of the Veterinary Teaching Hospital of
Helsinki University from January 2010 to December 2012. The outbreak period lasted
from November 2010 to January 2012. In late 2011 a small cluster of ST45 among
hospitalized patients contributed to an increase in incidence. From January 2012
onwards, the great majority of new MRSP findings have been detected in screening
targeted at risk patients on admission. In December 2012, the increase was not due
to a cluster, but was due to the detection of different types of MRSPs, mainly in
patients belonging to risk groups (Study I).

Trace-back analysis to one case in June 2010 (Figure 4) did not reveal any apparent
relationship with the outbreak. Screening of hospitalized patients and active case
findings revealed many new cases (Figure 3, Figure 4). Initial control measures in
late 2010 to early 2011 reduced the incidence, but new cases were detected in the
summer and fall of 2011. These led to the implementation of extensive control
measures. This seemed to disrupt and eventually end the outbreak. The
investigation was interfered by a small cluster of MRSP ST45 detected in late 2011
(Figure 4). The outbreak was declared over in January 2012, when no new MRSP-
positive patients were discovered in three consecutive screenings of hospitalized
patients.

A risk-based classification system was established during the outbreak
described in study I. This system is still in use at the VTH and has proven effective
in stopping further outbreaks of MRSP or other resistant bacteria. The system is
presented in Table 6.
Results and Discussion

Table 6  
The current risk based classification of patients at the Veterinary Teaching Hospital of Helsinki University and resulting measures.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria (any of the following)</th>
<th>Example of measures</th>
</tr>
</thead>
</table>
| High-risk patients   | - MRSP-positive  
                      - Has been hospitalized >24 hours or has signs of a hospital acquired infection.          | - Treated in cohort ward  
                      - Barrier nursing  
                      - Surgery at the end of the day  
                      - Disinfection of facilities  
                      - Infection sites cultured  
                      - Standard precautions*       |
| Medium-risk patients | - Has a history of recurrent ear or skin infection  
                      - Has a history of prolonged or numerous hospital visits or visits at other veterinary clinics  
                      - Has a history of prolonged or numerous antimicrobial treatments  
                      - Has been exposed to a patient with MRSP  
                      - Has had surgery elsewhere and has a surgical site infection  
                      - Has a suppurative wound infection | - Screened for MRSP  
                      - Treated in separate rooms reserved for medium-risk patients  
                      - Surgery at the end of the day  
                      - Standard precautions  
                      - Infection sites cultured       |
| Low-risk patients    | - All other patients                                                                                 | - All other rooms  
                      - Standard precautions           |                      |

*, Includes hand disinfection, a hygienic work routine, and the use of protective clothing in case of dirty procedures.

Nine new MRSP findings with the outbreak antibiogram were detected during the follow up period (February 2012 to December 2012). Seven of these had been hospitalized during the outbreak and were identified upon admission. The other two were not spatially or temporally connected to the outbreak. The total toll of cases connected to the outbreak was therefore 70. No new cases were detected among hospitalized patients, despite frequent screenings.

PFGE analysis supported the outbreak being due to the clonal spread of MRSP. Isolates clustered into one dominant pulsotype, A1 (n = 31), and four subtypes: A2 (n = 8), A3 (n = 6), and A4 (n = 1) with a one-band difference and A5 (n = 1) with a four-band difference (Figure 5). On the basis of the typing results for the three isolates, the strain responsible for the outbreak belonged to ST71 and harbored SCCmec II-III.
Figure 5  Dendrogram of 47 MRSP isolates with the outbreak antibiogram (see text). *Staphylococcus pseudintermedius* ATCC® 49444™ is displayed as a control. *Further characterized by multi-locus sequence typing and SCCmec typing.*
The outbreak strain (ST71-SCCmec II-III) caused a number of nosocomial infections in patients of the VTH, ranging from dermatitis to osteomyelitis. Most infections were surgical-site infections after non-elective procedures. In one case, colonization was followed by a urinary tract infection, complicated by urolith formation, which led to surgery. In another case, an MRSP infection was the most likely cause for euthanasia, but this could not be confirmed, since no autopsy was performed. Infections caused by the outbreak strain led to prolonged hospital treatment or additional surgical procedures being required to combat the infection. The majority of infections were treated without systemic antimicrobials, although amikacin was used if necessary. In one case, a urinary tract infection was treated with nitrofurantoin. The fact that MRSP infections were manageable without systemic antimicrobials is encouraging, and this approach could even be considered in infections caused by susceptible bacteria, provided that no systemic signs are present.

No common source for MRSP was identified during the outbreak. Several factors, however, suggest that this was a nosocomial outbreak, i.e. caused by HA-MRSP. An outbreak requires the cases to be spatially and temporally connected, which they were. Also, the patients displayed no evidence of MRSP on admission, and molecular characterization supported clonal spread. Furthermore, the epidemic curve (Figure 3) suggests nosocomial patient-to-patient transmission, and all infections were related to hospital care, as they were surgical-site infections or other infections that appeared after prolonged hospital treatment. It is also very unlikely that MRSPs of colonized patients were community acquired, since this MRSP type was very rare prior to the outbreak and no similar type of MRSP was observed among outpatients or specimens submitted from private clinics during the outbreak. In addition, many of our cases (n = 30) had given a negative MRSP result in former bacteriological specimens taken on or soon after first admission. Furthermore, as suggested by Kasai et al. (2016), there is evidence to support the idea that ST71 isolates, carrying SCCmec II-III, could be characterized as HA-MRSP due to its properties. A similar SCCmec-based classification has previously been suggested in human medicine with regard to MRSA (Klevens et al., 2007).

It is widely accepted that contaminated hands favor the spread of nosocomial pathogens (Weese, 2012). This may be efficiently controlled in hospitals by increasing the use of alcoholic hand rubs, as has been proven with MRSA (Lederer et al., 2009; Sakamoto et al., 2010). Furthermore, barrier nursing, as was implemented during our outbreak, has additionally been effective in reducing HA-MRSA (Perlin et al., 2013). Fomites may also play a role in the spread of hospital-associated pathogens, emphasizing the necessity of a clean environment and clothing (Boyce, 2007; Singh et al., 2013; Wilson et al., 2011). The environment was, however, probably not a major contributing factor in our outbreak, as only 1 out of 65 environmental specimens was positive for MRSP. It
is possible and even likely that the rapid response to the outbreak, along with the intensification of routine environmental cleaning procedures, reduced environmental contamination and effectively prevented environmental spread. In addition, cohorting, patient flow planning, emphasis on hand hygiene, barrier nursing, and prudent antimicrobial use were probably important (Weese, 2012).

Severe measures were required before control of the outbreak was gained. Reasons for the long duration of the outbreak are likely to be numerous. Studies have shown that MRSP ST71 is capable of efficient dissemination (Perreten et al., 2010) and biofilm formation (Osland et al., 2012). The initial lack of resources allocated for infection control probably contributed to the increased number of cases. In addition, new employees not familiar with the hygiene practices during the summer of 2011, combined with the lack of personnel due to holidays, may have contributed to the increase in incidence. On the other hand, more effective tracking of exposed patients and the appointment of a hygiene nurse seemed to reduce the number of new cases. Moreover, the cleaning and disinfection at the end of 2011 probably favored the cessation of the outbreak.

The role of colonized patients is likely to be underestimated in veterinary hospitals. While standard hygiene practices are effective in reducing the spread of MDR pathogens, extra precautions taken when treating identified carriers probably nullifies the risk of spread. Little evidence is available to support the use of a search-and-destroy policy, or decolonization treatment specifically for MRSP, as has been done with MRSA in some countries (Holzknecht et al., 2010; van Trijp et al., 2007). However, our search-and-isolate policy bears similar features. Since the outbreak, only sporadic cases of MRSP displaying the outbreak antibiogram have been identified, mainly among acknowledged risk patients, which indicates the success of the present policy. The total number of newly identified MRSP cases at the VTH has also significantly decreased since the outbreak.

### 5.2 Estimates for MRSP occurrence

The estimates of the occurrence of MRSP are based on the prevalence study on guide dogs, as well as crude estimations of prevalence by calculating the proportion of small animal clinical and screening specimens that revealed MRSP.

#### 5.2.1 Prevalence of MRSP among guide dogs (II)

Screening specimens were taken from 132 dogs, of which four were MRSP-positive (prevalence estimate for the population 3%, 95% CI: 1–8%). No MRSA-positive dogs were identified (prevalence estimate 0%, 95% CI: 0–3%). The breed
and sex of the sample population was representative of the target population, while the dog groups and age differed from that of the target population (Table 7).

Table 7  Comparison of demographics in the sample and target populations of the Finnish Guide Dog School’s dogs in study II.

<table>
<thead>
<tr>
<th></th>
<th>Sample population</th>
<th>Target population</th>
<th>Statistical difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 132</td>
<td>%*</td>
<td>n = 308</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65</td>
<td>49</td>
<td>158</td>
</tr>
<tr>
<td>Female</td>
<td>67</td>
<td>51</td>
<td>150</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>124</td>
<td>94</td>
<td>287</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Dog group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guide dog</td>
<td>56</td>
<td>42</td>
<td>187</td>
</tr>
<tr>
<td>Dog in training</td>
<td>68</td>
<td>52</td>
<td>103</td>
</tr>
<tr>
<td>Breeding dog</td>
<td>8</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.8 (0.2 – 13.9)</td>
<td>Median</td>
<td>2.1  (0.2 – 13.9)</td>
</tr>
<tr>
<td>Median</td>
<td>4.6 (0.2 – 13.9)</td>
<td></td>
<td>3.9</td>
</tr>
</tbody>
</table>

*Percentage values are rounded. Statistically significant P-values (<0.05) are bolded.

The observed prevalence of MRSP matched the predicted prevalence value. While no MRSA-positive dogs were discovered, it is important to note that with the CI used, the prevalence of MRSA may be as high as 3%. As seen in Table 7, dogs in training were overrepresented at the expense of guide dogs. This discrepancy is explainable by the ease of access, as dogs in training were more readily available for sampling at the GDS. This is also a reason for the difference in age, since dogs in training are younger than other dogs. The sample population represented the target population well in relation to breed and sex, and the targeted sample size (152) was close to the one obtained (132). The fact that the sample population was slightly skewed could, however, have impacted the results of the study.

Published studies regarding the prevalence of MRSP or MRSA in Finnish dogs are not available. Furthermore, only a few studies exist that have measured the prevalence of MRSP among specific animal groups. In a study of shelter animals by Gingrich et al. (2011) in Colorado, USA, the prevalence of MRSP in dogs was 3%, while the prevalence of MRSA in dogs was 0.5%. These numbers coincide quite well with our study. In Slovenia, the prevalence of MRSP (then MRSI) was
1.5% (3/200) in clinically healthy dogs in a community, while MRSA was not discovered (Vengust et al., 2006). Regarding veterinary hospitals, (Hanselman et al., 2008) reported a MRSP prevalence of 2.1% and MRSA prevalence of 0.5% in dogs upon admission in Ontario, Canada. A similar study conducted in Hannover, Germany, reported a higher MRSP prevalence (7.4%) (Nienhoff et al., 2011b). Furthermore, Boost et al. (2008) investigated the prevalence of S. aureus in dogs and their owners in Hong Kong. They found an MRSA prevalence of 0.7% among the dogs investigated, which falls into the 0–3% prevalence estimate in our study and corresponds well to our laboratory data. Further study is, however, needed to ascertain the prevalence of MRSP and MRSA in the average dog population, as well as subpopulations, to identify target groups for preventive measures.

5.2.2 Incidence and occurrence of MRSP among clinical specimens

The overall incidence (dogs and cats) of MRSP declined from 2011 to 2013 and remained stable from 2013 to 2015 (Figure 6). The same trend could also be seen for dogs and cats when considered separately. Out of all investigated canine clinical specimens (n = 9174), MRSP was isolated from 2.8% (2.5% if cats are included), a proportion similar to the MRSP prevalence in the Finnish guide dog population (3%). While this figure of 2.8% is not a true prevalence, it may be used as a crude approximation of the prevalence in an average canine population from which bacterial cultures have been taken, in order to design future prevalence studies. In feline specimens, the same figure was 0.4% (n = 1639).

![Figure 6](image_url)  
**Figure 6**  Yearly incidence of MRSP among clinical specimens from dogs (■), cats (▲) and overall (●) per thousand specimens at the Clinical Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Helsinki. Numbers next to data points indicate the total number of specimens for each animal species that year.
5.2.3 Occurrence of MRSP among risk patients (III)
MRSP from screening specimens from patients with identifiable risk factors, such as those listed in Table 6 (e.g. previous history of antimicrobial therapy or skin disease), was quite common, with an overall prevalence of over 9%. Screening specimen data for dogs and cats by year, are presented in Table 8.

Table 8
The proportion of MRSP per year among screening specimens from dogs and cats in Finland in 2011–2015.

<table>
<thead>
<tr>
<th>Year</th>
<th>MRSP%</th>
<th>n</th>
<th>MRSP%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011 June</td>
<td>9.2</td>
<td>347</td>
<td>11.5</td>
<td>52</td>
</tr>
<tr>
<td>2012</td>
<td>6.2</td>
<td>436</td>
<td>0.0</td>
<td>28</td>
</tr>
<tr>
<td>2013</td>
<td>10.2</td>
<td>551</td>
<td>0.0</td>
<td>24</td>
</tr>
<tr>
<td>2014</td>
<td>11.5</td>
<td>451</td>
<td>0.0</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>9.2</td>
<td>2237</td>
<td>3.0</td>
<td>197</td>
</tr>
</tbody>
</table>

*, the MRSP outbreak at the VTH was still ongoing in 2011; MRSP, methicillin-resistant S. pseudintermedius; n, number of specimens.

Prevalence data for risk patients are difficult to compare with existing studies, as no similar study has to our knowledge been conducted. As discussed above, the prevalence of MRSP varies quite substantially between studies and study populations. Our data are based on patients with identifiable risk factors for MRSP that have visited a veterinarian, either at the VTH or a private veterinary clinic in Finland. Thus, it would be inappropriate to compare the number to a population of healthy animals, such as those studied by Kjellman et al. (2015), or Gingrich et al. (2011). However, a study comparing the prevalence of S. (pseud)intermedius and MRSP, as well as other coagulase-positive staphylococci, in dogs with healthy and inflamed skin showed a similar difference compared to the prevalence estimates of our studies (Griffeth et al., 2008). In the study, 2% of healthy dogs (n=50) carried MRSP, while 7% of dogs with inflamed skin (n=59) carried MRSP.

5.3 Risk factors for MRSP (I–III)
In study I, four risk factors were identified among patients at risk during the MRSP outbreak at the VTH: skin lesions, antimicrobial treatment, and the cumulative number of days in the intensive care unit or in the surgery ward. In study II, although only a few animals were MRPS-positive, antimicrobial therapy and veterinary visits were risk factors among the Finnish guide dog population.
In study III, risk factors for a clinical *S. pseudintermedius* isolate being MRSP were ongoing antimicrobial or beta-lactam treatment during sampling, and being male. Among patients screened for MRSP, being a dog and receiving multiple antimicrobials during sampling were statistically significant risk factors for MRSP. Moreover, among both clinical and screening specimens in study III, the specimen originating from a private clinic increased the odds of *S. pseudintermedius* being MRSP. Results of the statistical analyses from each study are presented in Table 9, Table 10, Table 11, and Table 12. Note that in the tables, *n* refers to the number of dogs with the factor in question, while % refers to the proportion out of the total number of dogs from which data were available. For instance in Table 11 antimicrobial treatment data were available from 209 dogs among MRSP cases (84/0.402).
### Results and Discussion

#### Table 9  
**Risk factors associated with the acquisition of MRSP during the outbreak in the Veterinary Teaching Hospital of Helsinki University in 2010–2011 (Study I).**

<table>
<thead>
<tr>
<th>Binary variables</th>
<th>MRSP-pos ($n_{tot} = 55$)</th>
<th>MRSP-neg ($n_{tot} = 213$)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Wald</th>
<th>Adjusted OR (95% CI)</th>
<th>Wald</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender: M vs. F</td>
<td>30 (54.5)</td>
<td>96 (45.1)</td>
<td>1.46 (0.80-2.66)</td>
<td>0.212</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species: dog vs. cat</td>
<td>50 (90.9)</td>
<td>192 (90.1)</td>
<td>1.09 (0.39-3.06)</td>
<td>0.864</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidemiological data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin lesion</td>
<td>49 (89.1)</td>
<td>85 (39.9)</td>
<td>12.40 (5.06-30.37)</td>
<td>&lt;0.001</td>
<td>6.24 (2.30-16.97)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Antimicrobial treatment</td>
<td>52 (94.6)</td>
<td>130 (61.0)</td>
<td>11.07 (3.33-36.79)</td>
<td>&lt;0.001</td>
<td>3.80 (1.04-13.92)</td>
<td>0.0442</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td>45 (81.8)</td>
<td>67 (31.5)</td>
<td>9.81 (4.65-20.70)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporin treatment</td>
<td>21 (38.2)</td>
<td>24 (11.3)</td>
<td>5.10 (2.54-10.26)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin treatment</td>
<td>18 (32.7)</td>
<td>33 (15.5)</td>
<td>2.70 (1.3-5.2)</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity (1 vs. others)</td>
<td>46 (85.5)</td>
<td>8 (14.5)</td>
<td>2.84 (1.3-6.4)</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminopenicillin treatment</td>
<td>34 (61.8)</td>
<td>93 (43.7)</td>
<td>2.09 (1.14-3.85)</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment in ICU</td>
<td>41 (74.6)</td>
<td>126 (59.2)</td>
<td>2.02 (1.03-3.95)</td>
<td>0.039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proton pump inhibitor treatment</td>
<td>36 (65.5)</td>
<td>114 (53.5)</td>
<td>1.65 (0.89-3.06)</td>
<td>0.115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthopedic vs. soft tissue surgery</td>
<td>20 (44.4)</td>
<td>20 (30.3)</td>
<td>1.84 (0.83-4.08)</td>
<td>0.132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other antimicrobial treatment</td>
<td>12 (21.8)</td>
<td>32 (15.0)</td>
<td>1.58 (0.75-3.33)</td>
<td>0.229</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency surgery</td>
<td>6 (13.6)</td>
<td>9 (13.4)</td>
<td>1.02 (0.33-3.13)</td>
<td>0.976</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous variables</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodyweight</td>
<td>55 (20.7)</td>
<td>201 (19.2)</td>
<td>1.01 (0.99-1.03)</td>
<td>0.493</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>55 (4.8)</td>
<td>212 (4.9)</td>
<td>1.01 (0.92-1.10)</td>
<td>0.791</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidemiological data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days in ICU</td>
<td>55 (2.9)</td>
<td>213 (1.5)</td>
<td>1.39 (1.20-1.61)</td>
<td>&lt;0.001</td>
<td>1.3 (1.1-1.6)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Cum. days in surgery ward</td>
<td>55 (3.4)</td>
<td>213 (1.1)</td>
<td>1.28 (1.15-1.42)</td>
<td>&lt;0.001</td>
<td>1.1 (1.0-1.3)</td>
<td>0.0401</td>
</tr>
<tr>
<td>Cum. days in hospital (all wards)</td>
<td>55 (7.2)</td>
<td>213 (4.5)</td>
<td>1.15 (1.08-1.24)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days of proton pump inhibitors</td>
<td>36 (5.5)</td>
<td>114 (2.9)</td>
<td>1.08 (1.01-1.17)</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of surgical procedure (10 min change)</td>
<td>41 (203.2)</td>
<td>57 (170.9)</td>
<td>1.04 (0.99-1.10)</td>
<td>0.080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days in other wards</td>
<td>55 (2.0)</td>
<td>213 (2.6)</td>
<td>0.92 (0.81-1.05)</td>
<td>0.210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days antimic. given</td>
<td>55 (12.0)</td>
<td>213 (5.4)</td>
<td>1.01 (0.99-1.02)</td>
<td>0.364</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days of enroflox.</td>
<td>18 (6.5)</td>
<td>33 (4.7)</td>
<td>1.03 (0.96-1.11)</td>
<td>0.459</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days of aminopen.</td>
<td>34 (13.9)</td>
<td>93 (10.1)</td>
<td>1.00 (0.99-1.02)</td>
<td>0.481</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days cephalosp.</td>
<td>21 (5.3)</td>
<td>23 (4.1)</td>
<td>1.03 (0.93-1.15)</td>
<td>0.518</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum. days of other antimic.</td>
<td>12 (4.7)</td>
<td>32 (6.7)</td>
<td>0.98 (0.91-1.06)</td>
<td>0.614</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, Confidence interval. Statistically significant P-values (<0.05) are bolded.
Table 10  
*Risk factors associated with MRSP in the Finnish Guide Dog School population in 2014 (Study II).*

<table>
<thead>
<tr>
<th>Categorical variables</th>
<th>MRSP-pos $(n = 4)$</th>
<th>MRSP.neg $(n = 128)$</th>
<th>Unadjusted OR (95% CI)</th>
<th>Likelihood ratio $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender: F vs. M</td>
<td>3 75.0</td>
<td>62 48.4</td>
<td>2.48 (0.40-26.13)</td>
<td>0.338</td>
</tr>
<tr>
<td>Breed (Labrador retriever vs. other)</td>
<td>4 100.0</td>
<td>120 93.8</td>
<td>0.64 (0.06-86.75)</td>
<td>0.779</td>
</tr>
<tr>
<td>Dog bought vs. bred by school</td>
<td>0 0.0</td>
<td>13 10.2</td>
<td>0.95 (0.01-9.70)</td>
<td>0.973</td>
</tr>
<tr>
<td><strong>Dog group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding vs. training</td>
<td>2 50.0</td>
<td>6 4.7</td>
<td>8.39 (1.12-64.13)</td>
<td>0.012</td>
</tr>
<tr>
<td>Training vs. working</td>
<td>0 0.0</td>
<td>68 53.1</td>
<td>0.16 (0.00-2.01)</td>
<td></td>
</tr>
<tr>
<td><strong>Epidemiological data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of ear or skin disease</td>
<td>1 25.0</td>
<td>63 52.1</td>
<td>0.40 (0.04-2.48)</td>
<td>0.328</td>
</tr>
<tr>
<td>Antimicrobial treatment*</td>
<td>3 75.0</td>
<td>66 52.8</td>
<td>2.09 (0.33-21.99)</td>
<td>0.441</td>
</tr>
<tr>
<td><strong>Continuous variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSP-pos $(n = 4)$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>4 5.8</td>
<td>128 3.8</td>
<td>1.18 (0.92-1.49)</td>
<td>0.185</td>
</tr>
<tr>
<td>Number of veterinary visits*</td>
<td>4 9.8</td>
<td>128 4.9</td>
<td>1.23 (1.03-1.48)</td>
<td>0.025</td>
</tr>
<tr>
<td>Number of antimicrobial courses*</td>
<td>4 2.8</td>
<td>126 1.0</td>
<td>1.63 (1.04-2.55)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, Confidence interval using Firth’s bias adjustment (see text); *, for the past 12 months. Statistically significant $P$-values (<0.05) are bolded.
Results and Discussion

Table 11  *Risk factors associated with the discovery of MRSP among canine clinical S. pseudintermedius isolates taken in 2012–2015 (Study III).*

<table>
<thead>
<tr>
<th></th>
<th>MRSP</th>
<th>MSSP</th>
<th>Univariable logistic regression</th>
<th>Multivariable logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n_{tot} = 227)</td>
<td>(n_{tot} = 1501)</td>
<td>Unadjusted OR (95% CI)</td>
<td>Univariate P</td>
</tr>
<tr>
<td>Private clinic vs. Univ. Teach. Hosp.</td>
<td>192 84.6</td>
<td>1140 75.9</td>
<td>1.74 (1.19-2.54)</td>
<td>0.004</td>
</tr>
<tr>
<td>Gender: male vs. female</td>
<td>150 67.6</td>
<td>761 52.3</td>
<td>1.90 (1.41-2.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Deep lesion specimen</td>
<td>31 13.7</td>
<td>234 15.6</td>
<td>0.86 (0.57-1.28)</td>
<td>0.452</td>
</tr>
<tr>
<td>Superficial lesion specimen</td>
<td>173 76.2</td>
<td>1147 76.4</td>
<td>0.99 (0.71-1.37)</td>
<td>0.946</td>
</tr>
<tr>
<td>Urine specimen</td>
<td>8 3.5</td>
<td>81 5.4</td>
<td>0.64 (0.31-1.34)</td>
<td>0.238</td>
</tr>
<tr>
<td>Other specimens</td>
<td>15 6.6</td>
<td>39 2.6</td>
<td>2.65 (1.43-4.90)</td>
<td>0.002</td>
</tr>
<tr>
<td>Antimicrobial treatment during sampling</td>
<td>84 40.2</td>
<td>264 19.1</td>
<td>2.84 (2.09-3.87)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Antimicrobial groups: *

<table>
<thead>
<tr>
<th></th>
<th>Systemic beta-lactams</th>
<th>Multiple systemic antimicrobials</th>
<th>Other systemic antimicrobials</th>
<th>Topical antimicrobials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 71.4</td>
<td>1 1.2</td>
<td>15 17.9</td>
<td>8 9.5</td>
</tr>
<tr>
<td></td>
<td>150 57.3</td>
<td>6 2.3</td>
<td>63 23.9</td>
<td>40 15.3</td>
</tr>
<tr>
<td></td>
<td>1.87 (1.10-3.18)</td>
<td>0.51 (0.06-4.33)</td>
<td>0.25 (0.37-1.30)</td>
<td>0.58 (0.26-1.30)</td>
</tr>
<tr>
<td></td>
<td>0.022</td>
<td>0.541</td>
<td>0.252</td>
<td>0.189</td>
</tr>
<tr>
<td></td>
<td>1.87 (1.10-3.18)</td>
<td></td>
<td></td>
<td>0.90 (0.35-2.29)</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, Confidence interval; *, antimicrobial group variables were analyzed separately for patients that had received antimicrobials. Statistically significant P-values (<0.05) are bolded.
Antimicrobial therapy, either previous or during sampling, was implicated in some way as a risk factor in all studies. In addition, in studies I and II, a prolonged hospital stay and veterinary visits increased the risk of being MRSP (such data were not available for study III). The results from study I should not, however, be generalized to the overall population, as the study population consisted of patients hospitalized that were at risk of acquiring MRSP during an outbreak. Likewise, risk factor results from the studies are only valid for the population described, although the target population of study III was quite large, comprising small animals with infections from which a bacteriological specimen is obtained. It is interesting, although perhaps unsurprising, that the risk factors remained fairly similar between the studies. While several studies have evaluated the risk factors for MRSP infection, one risk factor has been documented above all: antimicrobial therapy (Eckholm et al., 2013; Huerta et al., 2011; Kasai et al., 2016; Nienhoff et al., 2011b). Specific antimicrobial drugs, such as clindamycin have also been indicated as increasing the risk of acquiring MRPS (Beck et al., 2012). Likewise, Kasai et al. (2016) found that treatment with beta-lactams was a risk factor for MRSP being recognized among clinical S. pseudintermedius isolates, similarly to the results of study III. Our results, as well as the results from the studies cited, are hardly surprising, as MRSP, commonly being MDR, is given a clear competitive advantage in hosts where the normal microbiota is suppressed by antimicrobial therapy.

Table 12  Risk factors associated with the discovery of MRSP among canine screening specimens taken during 2012–2015 (Study III).

<table>
<thead>
<tr>
<th></th>
<th>MRSP-pos (n=173)</th>
<th>MRSP-neg (n=1717)</th>
<th>Univariable logistic regression</th>
<th>Multivariable logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Private clinic vs. Univ. Teach. Hosp.</td>
<td>67</td>
<td>38.7</td>
<td>521</td>
<td>30.3</td>
</tr>
<tr>
<td>Gender: male vs. female</td>
<td>92</td>
<td>54.4</td>
<td>849</td>
<td>50.2</td>
</tr>
<tr>
<td>Antimicrobial treatment during sampling*</td>
<td>45</td>
<td>29.4</td>
<td>446</td>
<td>28.5</td>
</tr>
<tr>
<td>Systemic beta-lactams</td>
<td>20</td>
<td>13.1</td>
<td>219</td>
<td>14.0</td>
</tr>
<tr>
<td>Multiple systemic antimicrobials</td>
<td>10</td>
<td>6.5</td>
<td>56</td>
<td>3.6</td>
</tr>
<tr>
<td>Other systemic antimicrobials</td>
<td>7</td>
<td>4.0</td>
<td>108</td>
<td>6.3</td>
</tr>
<tr>
<td>Topical antimicrobials</td>
<td>6</td>
<td>3.9</td>
<td>45</td>
<td>2.9</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, Confidence interval; * antimicrobial treatment in general was not significant in the univariable analysis, and antimicrobial groups were therefore included in the model. Statistically significant P-values (<0.05) are bolded.
Furthermore, while hospitalized patients were at risk of acquiring MRSP during the hospital outbreak, it was curious to find that even among the small positive group in study II, frequent veterinary visits was a risk factor. Similar results have been reported by other studies, where previous hospitalization or frequent veterinary visits have been implicated (Eckholm et al., 2013; Lehner et al., 2014; Nienhoff et al., 2011b).

In study I, skin lesions were also associated with a higher risk of MRSP. While this variable did not differentiate whether the skin lesion was due to dermatitis, surgery, or something else, there is evidence to suggest that a chronic dermatological disorder is a risk factor for MRSP (Huerta et al., 2011). This is, however, probably due to long-term antimicrobial exposure, frequent veterinary visits (Lehner et al., 2014), and properties of the diseased skin (Simou et al., 2005), all of which could favor the acquisition of MRSP. There is also evidence to suggest that animals that carry MRSP are at a higher risk of getting an SSI, caused by any bacterium, post-surgery (Nazarali et al., 2015).

It was a surprising finding that S. pseudintermedius isolates being MRSP was more likely in specimens from private clinics compared to the VTH. This finding is given more credibility by the same result being obtained from the analysis of screening specimens. The reasons for this may be numerous. The VTH is not exclusively a referral hospital, but also treats first opinion patients. This probably dilutes some of the effect that chronically ill patients may be contributing to the prevalence of MRSP at the VTH. More importantly, the threshold to obtain bacteriological specimens early in the infection process is probably higher among private clinic veterinarians. The VTH has had a strict policy to obtain specimens in all cases of suspected bacterial infection since the MRSP outbreak in 2010–2011. It is also likely that screening criteria for risk patients are wider at the VTH compared to private clinics that, in our experience, mainly screen dermatological patients. This is a known risk group for MRSP, at least in part due to frequent antimicrobial therapy and veterinary visits (Lehner et al., 2014; Nienhoff et al., 2011b; Soares Magalhaes et al., 2010). Dissimilarities in patient populations may thus explain a difference in the proportion of MRSP, as has previously been reported (Beever et al., 2015).

Other risk factors identified in study III included gender, which is a risk factor that no study, to our knowledge, yet has reported. The reason for this is unclear. Studies have not found sex to be a predisposing factor for atopic dermatitis or food allergy (Bizikova et al., 2015; Nodtvedt et al., 2007; Verlinden et al., 2006). It is possible, or even likely, that some unknown variable could explain the result. Regardless, a German study investigating risk factors for MRSA in humans at a University hospital determined that male gender was a significant risk factor for MRSA acquisition. According to the authors, this may have been due to other factors that are associated with both the male gender and MRSA acquisition (confounding factors). These included diabetes mellitus-related terminal renal
failure, requiring dialysis, as well as invasive devices, such as bladder catheters. Our result may also be only due to chance, as gender was not observed to be a risk factor for MRSP in screening specimens.

It is also important to note that none of the studies was a longitudinal cohort study. Our results only show correlation, not causation, although causation may have been previously proven by other studies for some variables, such as antimicrobial treatment.

5.4 The role of cats

Five out of 55 cases (9%) in the VTH outbreak were cats. Cats were not separately analyzed in study I due to their low number and because it was deemed irrelevant, as the study population comprised patients of the VTH, not either species specifically. For study III, cats were omitted from the analyses due to their low number, as explained in section 4.7.3. Clinical specimens from dogs did, however, reveal more *S. pseudintermedius* and MRSP than those from cats. Furthermore, after 2011, not a single MRSP was isolated from feline screening specimens (n = 145), and only three were discovered in feline clinical specimens (n = 1509). It is therefore reasonable to conclude that the number of infected cats during the outbreak was disproportionately large due to the infection pressure at the hospital at that time. The post-outbreak results are unsurprising, as both *S. pseudintermedius* and MRSP colonization are less common in cats (Hanselman *et al.*, 2009; Ruscher *et al.*, 2009). Screening of cats for MRSP carriage, even if they have risk factors, is thus deemed unnecessary. Screening feline patients during MRSP outbreaks may, however, be important. It is worth noting that one study has indicated that cats (compared to dogs) have a higher risk of a clinical *S. pseudintermedius* isolate being MRSP (Lehner *et al.*, 2014). The number of isolates from cats in the study was, however, low.

5.5 Molecular epidemiology of MRSP

5.5.1 Molecular epidemiology (I–III)

The outbreak in study I was caused by the well-established global MDR MRSP clone ST71-SCCmec II-III (Perreten *et al.*, 2010). This was the most prevalent clone among MRSP isolates during 2010–2011 in Finland, although a shift in clonality has since occurred (see below). A parallel, smaller cluster of ST45 isolates was also detected in the later stage of the outbreak.
Results and Discussion

Characteristics of the four MRSP isolates of study II are compared in Figure 7: four were from the prevalence study (P-833, P-834, P-843, and P-860), two (P-853, and 2014-887) were from contact dogs of guide dog P-833 and three were isolates that had been preserved in 2012-2013 from other guide dogs (P-495, P-527 and P-781). All isolate except one (P-781) were multidrug resistant (MDR, resistant to ≥3 antimicrobial groups). The MRSP isolate of a contact dog, 2014-887, shared an identical antibiogram with the MRSP of guide dog P-833, but the other contact’s isolate (P-853) differed from these (Figure 7).

Figure 7  SmaI dendogram and antibiograms of the MRSP isolates investigated in the Finnish Guide Dog School MRSP study.

Dogs P-833, P-834, P-843, and P-860 were in the prevalence study, other dogs were contacts. Dogs P-833, P-853, and 2014-887 lived in the same household. OX, oxacillin; E, erythromycin; DA, clindamycin; SXT, sulphamethoxazole/trimethoprim; TE, tetracycline; FD, fucidic acid; ENR, enrofloxacin; CN, gentamicin; AK, amikacin; R, resistant; S, susceptible; NT, non-typeable; NA, not analyzed; Unk, the result from the SCCmec analysis of isolate P-781 was inconclusive (see text). The gray dashed line indicates the 85% cut-off value.

All isolates except for 2014-887, were available for molecular typing. Of the four isolates from the prevalence study, three (P-833, P-843, and P-860) were non-typeable by SmaI restriction, but gave identical Ascl restriction profiles (Appendix 1). Both SmaI and Ascl restriction patterns indicated that the other five isolates were not closely related. The four MRSP isolates from the prevalence study represented three different sequence types (Figure 7). Two were of ST45, while ST71 and ST402 had one representative each. The ST402 strain was a single locus variant (SLV) of ST45. The isolate from dog P-495 was an SLV of ST150. Isolates belonging to ST71 harbored SCCmec II-III. The ST45 and ST402 isolates were non-typeable by the SCCmec method used, as only the mecA gene was amplified in M-PCR-1. Isolate P-781, which belonged to ST403, gave an unusual result in the SCCmec analysis, as it seemed that products specific to the gene alleles ccrA2 and ccrA4 were amplified in M-PCR-1 (Appendix 2).

Out of the 362 MRSP isolates in study III (197 from clinical specimens, 165 from screening specimens), 279 were typeable using SmaI macrorestriction. These clustered into 19 (A to S) different clusters with four or more isolates (Appendix 3). Eighty-three isolates could only be typed by Ascl macrorestriction,
and formed two clusters (T and U) and one singleton in PFGE analysis (Appendix 4). In total, 87 isolates from 71 different PFGE clusters or singletons (SmaI or AscI) were investigated by MLST and SCCmec. Forty-two different STs were identified, including 19 new STs: 621 and 625–642 (https://pubmlst.org/spseudintermedius/). All SmaI non-typeable isolates belonged to CC45.

The proportion of isolates from each CC or ST changed from year to year, indicating increasing diversity in the MRSP population (Figure 8). All identified STs and their clonal and genetic relatedness are presented in Figure 9 A and B. Six STs grouped together with previously described CCs (CC45 and 258) (Duim et al., 2016). STs in the CC258 group were scattered in the sequence comparison tree, while STs in the CC45 group were more alike.

![Figure 8](image-url) Proportion of clonal complexes or sequence types of methicillin-resistant Staphylococcus pseudintermedius by year in 2010–2014. The data are based on the extrapolation of MLST analysis results to the corresponding PFGE cluster. Numbers on top of the columns indicate the number of isolates. Other, miscellaneous sequence types.
Figure 9 (previous page) Genetic relationship of methicillin-resistant *Staphylococcus pseudintermedius* in Finland (2010 to 2014). A: goeBURST analysis conducted at the double-locus variant level, with triple-locus variants (connected with gray dashed lines) added to show further relatedness. Line numbers and shading indicate the number of differing loci between sequence types. CC, clonal complex. Gray areas highlight CCs. Gray boxes with black text, group founder; black boxes with white text, sub-group founder; white boxes with black text, common node; gray boxes with white text, triple-locus variant. B: A phylogenetic tree based on the alignment of all MLST genes of each ST. Only posterior probabilities >0.7 are shown. Gray shades indicate clonal complex groups as assigned by goeBURST analysis. *, clonal complex founder; †, single-locus variant; ‡, double-locus variant.

Four different SCCmec types, covering 60 out of the 87 (69%) investigated isolates, were identified. Twenty-seven isolates (31%) were non-typeable, either due to a lack of a PCR product or because the result did not match SCCmec types I–VI (Appendix 5). The number of isolates found to carry each SCCmec, as well as the STs in which these were found, are presented in Table 13.

Table 13 *The number of MRSP isolates with each identified SCCmec, as well as the sequence types associated with them.*

<table>
<thead>
<tr>
<th>SCCmec</th>
<th>Number of isolates</th>
<th>Sequence Type(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>1</td>
<td>498</td>
</tr>
<tr>
<td>II-III</td>
<td>19</td>
<td>71</td>
</tr>
<tr>
<td>IV</td>
<td>37</td>
<td>258, 342, 413, 415, 475, 561, 592, 621, 625, 626, 630, 631, 632, 633, 634, 635, 638, 639, 640, 641</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>183, 404, 642</td>
</tr>
<tr>
<td>Non-typeable</td>
<td>27</td>
<td>21, 41, 45, 84, 121, 150, 152, 263, 298, 305, 402, 403, 561, 628, 636, 637</td>
</tr>
</tbody>
</table>

The identification of 23 previously identified and 19 new STs is a testament to the diverse nature of the MRSP population in Finland. A changing trend was evident, as the population diversified during the time frame investigated (Figure 8). It is, however, important to note that in 2010–2011, many isolates originated from the VTH outbreak. Before the outbreak, MRSP was a very uncommon finding in the VTH, although previously published surveillance data indicate that MRSP arrived in force in Finland in 2009, when a sharp increase in the proportion of MRSP was observed (FINRES-Vet, 2015). A Swedish study (isolates from 2008 to mid-2010) revealed a fairly homogeneous MRSP population, with 96% (216/226) of the isolates representing the predominant European lineage, ST71 (Borjesson et al.,
This clone was also the most common ST in Finland in 2010–2011, but has diminished since (Figure 8). As in our data, a change in the MRSP population has also been observed in Sweden since 2010 (SWEDRES-SVARM, 2016). There, 20 out of 58 MRSP isolates (34%) were ST258 in 2015. CC258 was the third most common CC in our study, and was the largest clone in Norway in a recent study (Kjellman et al., 2015). Additionally, CC258 has recently been reported as a major CC in both the Netherlands (Duim et al., 2016) and Denmark (Damborg et al., 2016). These studies also reported a marked diversity in the MRSP population in recent years. It thus appears that ST71 has lost its sustainability and CC258 is only filling the gap as a more successful lineage. ST71 carries SCCmec II–III, which has only been found in related STs and ST354 (Damborg et al., 2016; Ishihara et al., 2016). The immobility of this element may be an underlying reason for the demise of the clone. In contrast, our data indicate that SCCmec IV, in particular, is readily transferred between different clones, or is received from other staphylococci, as it was identified in 20 different STs. The acquisition of SCCmec elements by MSSP from MRSP, CoNS, or MRSA may explain the plethora of STs in MRSP. Our findings indicate that the epidemiology of MRSP is changing; clonal spread is becoming less significant and the spread of SCCmec elements is more common. This could make it more difficult to control the spread of MRSP.

Contrary to CC71, CC45 has maintained a steady proportion, with an over 20% annual share of the MRSP isolates in 2011–2014. Representatives of this CC have been detected at least in Sweden (SWEDRES-SVARM, 2015), the Netherlands (Duim et al., 2016), and in Israel and Thailand (Kadlec et al., 2016; Perreten et al., 2013), but has been rather rare in reports from Europe (Duim et al., 2016; SWEDRES-SVARM, 2015). Representatives of ST45 are typically non-typeable by SmaI PFGE (Perreten et al., 2013), as was the case in our study. Interestingly, 80% (66/83) of CC45 isolates showed the same basic antibiogram, only being susceptible to trimethoprim/sulfamethoxazole and fusidic acid (Appendix 4). The distribution of STs among clinical isolates and screening isolates was similar, with ST71 being the most common and ST45 being the second most common ST in both groups (data not shown).

The sequence alignment and eBURST analyses grouped the identified STs quite differently. For example, STs 258 and 413, single-locus variants, were placed in different groups in the maximum likelihood tree (6 nucleotide differences in one locus), while ST638, a double-locus variant to ST258, was deemed nearly identical to ST258 (4 nucleotide differences in two loci) in the maximum likelihood tree (Figure 9). Performing eBURST analysis of MLST data has become common when analyzing the clonality of MRSP. However, it does appear to be a crude way of assigning genetic relatedness. A single locus, e.g. cpn60 alleles 1 and 10, may have 26 single nucleotide differences. All other alleles being identical, two isolates would be considered single-locus variants, and be assigned the same CC. On the other hand, two isolates that are triple-locus variants, but only by one
nucleotide in each locus (e.g. *ack* 1 and 2, *fdh* 1 and 5, and *sar* 1 and 7), would not be assigned to the same CC, even though the total number of nucleotide differences is much smaller (3 versus 26). It could be beneficial to determine genetic relatedness based on the actual sequences, as was done by Kjellman *et al.* (2015), rather than by comparing combinations of allele numbers, as much resolution is lost in the latter method. It is, however, likely that whole genome sequencing will replace eBURST analysis once its costs decrease.

The epidemiology of MRSP is complex, being a combination of clonal propagation and the spread of resistance genes, although a trend of change is clearly seen. While the strain that caused the outbreak at the VTH was multiresistant, the resistance was predictable, and any suspicion of MRSP infection could therefore be swiftly responded to. The dispersal of resistance genes across a plethora of STs has made such predictions impossible, as some isolates may be resistant to nearly all tested antimicrobials, while others are solely resistant to methicillin.
5.5.2 Geographical distribution of MRSP in Finland (unpublished)

Specimens with *S. pseudintermedius* were received from all except one region of the Finnish mainland: Lapland. The number of specimens yielding *S. pseudintermedius* ranged from 18 to 1651 among different regions. Southern Finland was most represented, both in the number of clinical specimens (n = 1651) and isolates investigated by molecular methods (n = 330). A summary of the different CCs and STs per region is presented in Figure 10. The proportion of MRSP among all *S. pseudintermedius* from each region is presented in Figure 11.

![Figure 10](image)

*Figure 10* Distribution of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) clonal complexes and sequence types (ST) per region in Finland and the proportion of MRSP among clinical *S. pseudintermedius* isolates (screening specimens excluded) from the whole study period. The data are based on the extrapolation of MLST analysis results to the corresponding PFGE cluster. The numbers in parentheses indicate the number of isolates investigated by molecular methods (including screening specimens) for each region. Only one isolate from Northern Finland was investigated, and it belonged to ST640.
While most small animals in Finland do live in the southern parts, this region is clearly overrepresented in this study. It is, however, surprising to see that despite this, the proportion of MRSP is very similar in specimens from different regions. Also, the composition of the MRSP population is very comparable among the two regions where most isolates were investigated, Southern and South-Western Finland. More information is, however, required before any inference may be made on the distribution of MRSP in Finland. Nevertheless, based on the
available data, it would seem reasonable to expect MRSP to be distributed fairly equally nationwide.

5.6 The changing role of MRSP

During the MRSP outbreak at the VTH (2010–2011), it seemed clear that MRSP was a healthcare-associated (HA) rather than a community-associated (CA) pathogen. However, it may be challenging to classify infections into HA-MRSP and CA-MRSP in veterinary medicine. One problem is the lack of commonly accepted definitions for HA infections. In humans, MRSA infections are classified as HA or CA, while the former is subdivided into community-onset and hospital-onset (Klevens et al., 2007). Community-onset HA-MRSA is defined as a case with at least one of the following risk factors: the presence of an invasive device at time of admission; a history of MRSA infection or colonization; a history of surgery, hospitalization, dialysis, or residence in a long-term care facility in the previous year. Hospital-onset cases are defined as cases with positive culture results from a normally sterile site obtained >48 h after admission, and may have ≥1 of the community-onset risk factors (Klevens et al., 2007). CA-MRSAs are defined as cases that do not have the previous risk factors. Hospital stays are usually shorter for pets than humans, especially for outpatient surgery (day surgery). As such, it would be expected to see far more community-onset HA-MRSP, as infection usually takes a couple of days to develop. It is therefore questionable whether post-procedure infections are correctly classified as HA.

While the number of HA-MRSP cases has declined, at least at the VTH (data not shown), CA-MRSP is probably on the rise. The shift in clonality is one piece of evidence for this; if MRSP would be predominantly HA, one would perhaps expect to see a small number of different clones appearing continuously in the population. Indeed, as previously noted, it has been suggested that CC71 isolates be classified as HA-MRSP outright (Kasai et al., 2016). Moreover, HA-MRSP would probably manifest itself as the cause of numerous HA infections, which are currently quite rare at the VTH. Nonetheless, a possible shift from HA to CA spread increases the likelihood of low-risk patients (i.e. patients without known risk factors) becoming at least contaminated, if not colonized, with MRSP. A near identical development to this was seen with MRSA in human healthcare, as it began as a HA problem, but soon began spreading in the community (Holzknecht et al., 2010; Woodford and Livermore, 2009). The shift in the method of spread does not mean that veterinary facilities are irrelevant to the spread of MRSP. They still retain a role as hubs where animals and bacteria of different origins, as well as selective pressure by antimicrobials, are present. The importance of infection control in veterinary premises should therefore be stressed as an imperative means of preventing the spread of resistance.
5.7 Antimicrobial resistance

5.7.1 Resistance among clinical *S. pseudintermedius* isolates (III)

Results were available from a total of 1958 clinical *S. pseudintermedius* isolates. Of these, 1471 (75%) were from specimens from private clinics, while 487 were from the VTH. The isolates were mainly from dogs, comprising 1928 isolates (98%), while 18 isolates (0.9%) originated from cats. One *S. pseudintermedius* isolate was from a guinea pig. In eleven cases (0.6%), the species had not been recorded. The majority of specimens (n = 1507; 77%) were obtained from superficial sites, such as ears and skin, while 284 (15%) were from deep lesions (e.g. deep wounds, abscesses, or synovial fluid). The bacterium was also discovered from urine (n = 98; 5%), respiratory specimens (n = 6; 0.3%), and blood cultures (n = 2; 0.1%). The rest (n = 61; 3%) were from cultured agar plates that had been sent to the laboratory for species identification and susceptibility testing.

The overall proportion of oxacillin (methicillin) resistance among *S. pseudintermedius* was 14% (n = 266). While this overall percentage is lower than that in 2011 (17%), it is important to note that the MRSP outbreak at the VTH probably contributed significantly to the number that year. Non-susceptibility data for all tested antimicrobials for MRSP, MSSP, and overall, are presented in Table 14. The proportion of non-susceptible isolates per year varied only slightly for each antimicrobial, and no statistically significant trends were detected (Figure 12).
Results and Discussion

Table 14  Antimicrobial non-susceptibility among clinical Staphylococcus pseudintermedius isolates from mid-2011 to the end of 2015 in Finland.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MRSP % non-S</th>
<th>95% CI</th>
<th>n</th>
<th>% non-S</th>
<th>95% CI</th>
<th>n</th>
<th>% non-S</th>
<th>95% CI</th>
<th>All (2015)† % non-S</th>
<th>95% CI</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic panel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin*</td>
<td>85.7</td>
<td>80.8-89.6</td>
<td>265</td>
<td>22.2</td>
<td>20.2-24.3</td>
<td>1678</td>
<td>30.8</td>
<td>28.8-32.9</td>
<td>1947</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin*</td>
<td>85.7</td>
<td>80.8-89.6</td>
<td>266</td>
<td>21.8</td>
<td>19.9-23.9</td>
<td>1678</td>
<td>30.5</td>
<td>28.5-32.6</td>
<td>1949</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>24.5</td>
<td>19.5-30.2</td>
<td>265</td>
<td>24.3</td>
<td>22.3-26.4</td>
<td>1677</td>
<td>24.4</td>
<td>22.5-26.4</td>
<td>1946</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin*</td>
<td>100</td>
<td>98.2-100</td>
<td>266</td>
<td>0</td>
<td>0.0-0.3</td>
<td>1682</td>
<td>13.7</td>
<td>12.2-15.3</td>
<td>1948</td>
<td></td>
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<tr>
<td>Tetracycline*</td>
<td>74</td>
<td>68.2-79.1</td>
<td>265</td>
<td>33.3</td>
<td>31.1-35.6</td>
<td>1675</td>
<td>38.8</td>
<td>36.6-41.0</td>
<td>1944</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/ Sulfamethoxazole*</td>
<td>47.7</td>
<td>41.6-53.9</td>
<td>266</td>
<td>6.1</td>
<td>5.0-7.4</td>
<td>1680</td>
<td>11.8</td>
<td>10.4-13.3</td>
<td>1951</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Extended panel                  |              |        |    |         |        |    |         |        |                     |        |    |
| Amikacin                       | 0            | 0.0-2.1 | 219 | 0       | 0.0-0.9 | 547  | 0       | 0.0-1.2 | 393                 |        |    |
| Chloramphenicol*               | 46.9         | 29.5-65.0 | 32  | 15      | 10.6-20.8 | 207  | 18.4    | 13.7-24.2 | 228                |        |    |
| Doxycycline*                   | 28.8         | 18.6-41.4 | 66  | 4.7     | 2.8-7.7  | 342  | 6.1     | 4.0-9.1 | 392                 |        |    |
| Enrofloxacin*                  | 50.4         | 44.0-56.8 | 248 | 2.6     | 1.7-4.0  | 793  | 7.3     | 5.0-10.4 | 395                |        |    |
| Gentamicin*                    | 44.8         | 38.5-51.2 | 248 | 2.5     | 1.6-3.9  | 795  | 6.6     | 4.4-9.6 | 395                |        |    |

*, Statistically significant (P < 0.001) difference between MRSP and MSSP; †, consistent data only available for 2015, as the extended panel was only investigated for MRSP or otherwise MDR isolates prior to this; MRSP, methicillin-resistant S. pseudintermedius; MSSP, methicillin-susceptible S. pseudintermedius; non-S, non-susceptible; n, number of isolates.

Figure 12  Non-susceptibility percentages by year with 95% CI for clinical Staphylococcus pseudintermedius isolates in Finland from mid-2011 to the end of 2015. Cochran-Armitage trend test P-values were: 0.14 for CLI (clindamycin); 0.14 for ERY (erythromycin); 0.86 for FUS (fusidic acid); 0.12 for OXA (oxacillin); 0.22 for SXT (sulfamethoxazole/trimethoprim); 0.40 for TET (tetracycline).
A complete basic panel antibiogram was recorded for 1932 (99%) isolates. Of these, 17% (321 isolates) were MDR. The most common MDR profile (78/321; 24%) was lincosamides/macrolides–oxacillin–trimethoprim/sulfamethoxazole–tetracycline. Twelve isolates were non-susceptible to all six antimicrobials investigated, while 36% (700/1932) were fully susceptible.

Non-susceptibility to antimicrobials was significantly higher among MRSP isolates than MSSP isolates for all antimicrobials, except for fusidic acid, for which non-susceptibility was around 24% in both. Similar findings apply to MRSA and MSSA (Livermore et al., 2002). Resistance to fusidic acid among S. pseudintermedius has varied between studies. In Sweden, 20% of isolates were resistant to fusidic acid in 2015 (MIC ≥ 1 mg/L) (SWEDRES-SVARM, 2016), which is similar to our results. In Norway, nearly half of S. pseudintermedius isolates investigated were fusidic acid resistant (MIC ≥1 mg/L) (Norstrom et al., 2009). On the other hand, an Italian study did not find fusidic acid resistance in S. (pseud)intermedius (Vanni et al., 2009).

A recent report from Sweden details resistance among S. pseudintermedius collected from skin lesions (SWEDRES-SVARM, 2016). Although our data also include isolates from other sources, the difference in oxacillin resistance, in particular, is exceptional, being 2% in the Swedish data and 14% in our data for 2015. Similar differences can be seen among other tested antimicrobials, which is concerning. These differences may reflect contrasts in the use of antimicrobials and infection control policies in these countries. This may be reflected in the high proportion of animals receiving antimicrobials in the different studies. In study I, over 90% of MRSP-positive and over 60% of MRSP-negative patients had received antimicrobials. In study II, over 50% of the dogs enrolled had been treated with antimicrobials within the past year, and in study III, 22% of animals from which S. pseudintermedius was isolated from a clinical specimen were receiving antimicrobials during sampling. Among patients from which screening specimens had been obtained, this number was 29%. While it is likely that some bias is present, since animals from which specimens were obtained had visited the veterinarian at least once (except for guide dogs, from which specimens were taken at the Guide Dog School), these high numbers raise concerns about the use of antimicrobials in small animals in Finland. National reports confirm that the usage of antimicrobials in Sweden is lower than in Finland, and that Swedish dogs are prescribed antimicrobials with a more narrow spectrum (FINRES-Vet, 2015; SWEDRES-SVARM, 2016).

Due to the high resistance towards commonly used antimicrobials in S. pseudintermedius in Finland, clinicians are strongly encouraged to take specimens for bacterial cultures early in the disease process to ensure the efficacy of the intended treatment. Moreover, the use of local, or non-antimicrobial therapy should be emphasized as a viable alternative to many infections. These issues are also discussed in the newly published national guidelines on the use of
Results and Discussion

antimicrobials in animals (Finnish Food Safety Authority and the Faculty of Veterinary Medicine, 2016). In addition, since *S. pseudintermedius* is a common finding in infections associated with dermatological diseases, it is vital that any underlying disease process is properly controlled to avoid the unnecessary use of antimicrobials. More research is required to determine the value of antimicrobial therapy in patients when underlying conditions have been controlled. Furthermore, the high resistance among *S. pseudintermedius* could warrant making MRSP a notifiable animal disease in Finland, as it is in Sweden (SWEDRES-SVARM, 2016).

5.7.2 Oxacillin susceptibility histograms and production of beta-lactamases (unpublished)

The distribution of oxacillin ZOI diameters for 2011 and 2015 is presented in Figure 13.

![Figure 13](image)

**Figure 13** Zone diameter distributions for oxacillin in 2011 and 2015 for *Staphylococcus pseudintermedius* at the Clinical Microbiology Laboratory of the Faculty of Veterinary Medicine in 2011–2015. The vertical dashed line designates the breakpoint for susceptible isolates.

In 2007, Jones *et al.* (2007) published data on the susceptibility of *S. (pseud)intermedius*. The data indicated that the number of oxacillin-resistant isolates clearly increased in 2001–2005. The study also divided the oxacillin data into highly resistant (ZOI for oxacillin ≤ 10 mm), resistant (ZOI 11–17 mm), and susceptible (ZOI ≥ 18 mm). The proportion of highly resistant isolates remained fairly constant, 20–30%. The same cannot be said for our data (Figure 13). In 2011, roughly 80% of MRSP isolates were highly resistant to oxacillin. Four years later,
the same number was roughly 36%, a clear shift in the distribution of zone diameters. Note, however, that many isolates in 2011 belonged to the VTH outbreak clone. It is likely, although not studied here, that the shift in the distribution reflects the shift in clonality, as a larger number of different clones would spread out the histogram among methicillin-resistant isolates. The shift in oxacillin susceptibility may also make it more difficult to identify carriers of MRSP with the screening method used in our studies, as an increased ZOI for oxacillin indicates a decrease in oxacillin MICs of the MRSP population.

There were 421 MSSP isolates in the 2016 laboratory data. Beta-lactamase production was tested in 252 cases (60%). Out of these, 231 (92%; CI95% 88–94%) were beta-lactamase positive. The proportion of beta-lactamase producers among MSSP isolates was somewhat higher in our data than that of a Norwegian study, where only 70% of S. pseudintermedius isolates produced beta-lactamase, i.e. were resistant to penicillin (Norstrom et al., 2009). Similar resistance rates to penicillin (72%) have been seen in Australia (Bean and Wigmore, 2016). However, a Lithuanian study determined, by PCR detection of the blaZ gene, that 94% of S. pseudintermedius isolates from diseased dogs were resistant to penicillin (Ruzauskas et al., 2016). This number is very similar to our result. Interestingly, in a multinational European study, penicillin resistance in S. pseudintermedius was reported to be only ~20% (Ludwig et al., 2016). This was, however, determined by a MIC method using breakpoints based on human data.

Due to the high proportion of MSSP isolates being resistant to beta-lactamase susceptible antimicrobials (e.g. penicillin, ampicillin, and amoxicillin), the use of these drugs in the treatment of skin infections cannot be recommended. On the other hand, the use of beta-lactamase-resistant drugs, such as amoxicillin-clavulanic acid or cephalexin, may increase the risk of the patient becoming an MRSP carrier.

5.8 Limitations of the study

As with any study, there are limitations to consider. In study I, the quality of the data was dependent on how well information had been recorded in the patient management software. This type of bias is, however, expected to be equally distributed among cases and controls. Additionally, information related to patient care, such as the number of times the animal was handled or the exact placing of the patient (e.g. cage number), was unavailable. Such data could help in understanding the dynamics of the outbreak. It may be considered a limitation that not all patients could be screened for MRSP upon admission. As a result, it cannot be ruled out that some of the patients may have had MRSP prior to admission. During an outbreak, however, it is not realistic, nor necessary, to screen every patient in order to determine their admission status. As discussed
above, the likelihood of a community acquired MRSP ST71 was very low when all evidence was considered.

In study III, the information on risk factors, such as antimicrobial therapy, was prone to reporting bias, as this information is more likely to be reported if the animal is receiving treatment. This is, however, unlikely to have impacted on our results, as the minimum data coverage for risk factors was 88% in that study.

Also, due to the imperfect sensitivity of the MRSP screening method used in the studies, some degree of misclassification is likely to have occurred. Test specificity was probably high, as all isolates were characterized after isolation. Currently, no reference standard for the screening of MRSP is available. Other studies have, however, used similar methods to the one utilized here (Gomez-Sanz et al., 2011; Paul et al., 2011). Studies regarding MRSA in humans or livestock, where similar methods have been utilized, have reported sensitivities of up to 98% (Pletinckx et al., 2012; Veenemans et al., 2013; Verkade et al., 2011a; Verkade et al., 2011b). However, as indicated in section 2.7, commercial MRSA-selective agars may contain cefoxitin as the selective antimicrobial. The outbreak strain was highly resistant to oxacillin (MIC >256 mg/L), and was therefore also likely to have been highly resistant to cefoxitin (Bemis et al., 2009; Bemis et al., 2006; Bemis et al., 2012). Based on our internal evaluation of the method used, the limit of detection is approximately 10 colony forming units, provided that MRSP has an oxacillin MIC ≥4 mg/L. Also, the extension of the incubation time to 48 hours for initially negative plates increases the sensitivity (personal observation), and has revealed isolates with MICs as low as 2 mg/L. It is therefore unlikely that a significant amount of misclassification would have occurred.

The sensitivity of the method is also dependent on the sampling. The sample sites were selected based on two criteria: 1) sites reported in the literature to give a high yield of MRSP and 2) convenience. Sampling sites vary between studies, but the nose, mouth, and perineum appear in many (see section 2.7 for a detailed review). A study by Windahl et al. (2012) reported that the pharynx, closely followed by the perineum, yielded the most MRSP isolations of the positive sample occasions. The collection of swabs from the pharynx is, however, difficult (and even dangerous) and can cause discomfort to the dog. Thus, this sample site was omitted.

Regarding the molecular epidemiology, it is worth noting that specimens mainly originated from Southern Finland and may thus not reflect the resistance situation in the entire country. On the other hand, the proportion of private clinic specimens rose from 16% in 2011 to 47% in 2015, with nearly 200 submitting clinics, which increased the geographical coverage. Furthermore, PFGE was used as a screening method to find candidates for MSLT and SCCmec typing without having to type all MRSP isolates, which was not feasible. It is therefore possible that there are other, as yet unidentified, STs among the isolates. However, apart
from one instance where multiple isolates had been typed from a PFGE cluster, all belonged to the same CC.

In the susceptibility data of study III, there may be more than one isolate from the same animal, which may have caused bias in the data. Such bias is, however, likely to be minimal due to the large number of isolates, and because such isolates would probably be distributed evenly among susceptible and resistant populations and over the years. For molecular data, only the first MRSP isolate of an animal was stored.
6 Conclusions

The use of strict hygiene protocols and barrier nursing are necessary in combating an MRSP outbreak.

MRSP has a low prevalence (3%) among guide dogs in Finland, and a similar proportion (2.5%) of clinical specimens from small animals revealed MRSP. Among screening specimens taken from patients with risk factors for MRSP, over 9% carry the bacterium.

The low number of clinical MRSP isolates from feline specimens, and the lack of MRSP-positive cats in screening specimens, indicates that the targeted screening of feline patients is unnecessary and unlikely to be cost effective.

Antimicrobial therapy, a prolonged hospital stay, and frequent veterinary visits are identifiable risk factors for MRSP.

Antimicrobial resistance for several antimicrobials among Finnish S. pseudintermedius isolates is high and a cause for concern, especially as 14% of isolates are methicillin resistant. This emphasizes the need to obtain a bacteriological specimen whenever antimicrobial therapy is indicated. Moreover, the use of non-antimicrobial therapy for infections should be considered whenever feasible.

The epidemiology of MRSP has shifted away from a primarily clonal spread to being spread both clonally and through the transfer of resistance genes.

A worryingly high proportion of patients in the studies were receiving or had received antimicrobials. Decisive action is required to reduce the consumption of antimicrobials in small animals.
7 Future aspects

There are still many important aspects to investigate regarding MRSP in Finland. The most important need is undoubtedly to investigate the actual prevalence of MRSP in dogs, as well as in specific risk populations, such as dermatological patients. This should preferably be done with a new, more sensitive method than the one used in this study, perhaps utilizing PCR for rapid screening of specimens that need to be cultured. Such a project is already underway.

In addition, more information is needed on how long animals carry MRSP and to establish guidelines on how many consecutive negative screening specimens are required to declare an animal free of MRSP. Data on this have already been collected by our research team. It would also be beneficial to determine whether decolonization therapy for MRSP is indicated for small animal patients prior to surgical intervention, or whether such therapy is effective at all.

Furthermore, to maintain a grasp of the MRSP situation in Finland in the future, it would be preferable if molecular analyses were carried out yearly for representative MRSP isolates. Moreover, making MRSP a notifiable animal disease would help in mapping the extent of Finland’s MRSP situation. In addition, more frequent, preferably annual, reports on the resistance patterns and proportions for common animal pathogens could help clinicians adapt their therapeutic regime to immediately select an appropriate antimicrobial. This would hopefully also bring forth the problem of resistance. One possibility would be to annually publish resistance data in a concise form in the Finnish Veterinary Journal.

It could also be beneficial to explore the usefulness of novel methods, such as MALDI-TOF, as tools for rapidly identifying and typing MRSP isolates. In addition, investigating the clinical efficacy of antimicrobial treatment for different infections would be important in order to reduce the consumption of antimicrobials.
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To my mother Chrissie and my godmother Nea: you supported me in my efforts to become a veterinarian; hence, I would not be writing this were it not for you. Thank you for all you have done for me through the years. I wish to expressly thank you for an excellent upbringing. It is something that comes in handy when you are outnumbered ten to one by women in the workplace.

In conclusion, I want to thank Henna, the love of my life, for your love, support and encouragement. You have stood by me through the good and the bad, and you found time for me despite your long hours at the office. We make a great team, you and I, and there’s no “I” in “team”. There is a “me”, though, if you jumble it up.

Helsinki 30.5.2017
Thomas Grönthal


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Wilson, A.P., Smyth, D., Moore, G., Singleton, J., Jackson, R., Gant, V., Jeantes, A., Shaw, S., James, E., Cooper, B., Kafatos, G., Cookson, B., Singer, M., Bellingan, G., 2011. The impact of enhanced cleaning within the intensive care unit on contamination of the near-patient environment with


Appendix 1

Ascl PFGE dendogram of the MRSP isolates investigated (Study II).
All isolates were also investigated using SmaI restriction (see text).
Appendix 2

Gel image of the products of isolate P-781 and positive controls after SCCmec PCR. (Study II)

ctr, control; SCCmec I ctr, Staphylococcus aureus NCTC 10442; SCCmec II ctr, Staphylococcus aureus ATCC® BAA-1720™; SCCmec III ctr, Staphylococcus aureus ATCC® BAA-43™, SCCmec V ctr, Staphylococcus aureus JCSC 6944; SCCmec VI ctr, Staphylococcus aureus ATCC® BAA-42™.
Appendix 3

<table>
<thead>
<tr>
<th>Isolate</th>
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<th>Clinic</th>
<th>Date of isol.</th>
<th>Specimen</th>
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<th>RGA</th>
<th>MLST</th>
<th>SCCmec</th>
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<td>Dog</td>
<td>VTH</td>
<td>2013-12-18</td>
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<td>RRRRRS</td>
<td>SSS</td>
<td>ST 628</td>
<td>NT</td>
<td></td>
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<td>PC</td>
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<td>SS-</td>
<td>ST 71</td>
<td>II-III</td>
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<td>SS-</td>
<td></td>
<td></td>
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<td>SS-</td>
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Sma1 PFGE dendrogram with antimicrobial resistance profiles of methicillin-resistant *Staphylococcus pseudintermedius* isolated at the Laboratory of Clinical Microbiology of the Faculty of Veterinary Medicine, University of Helsinki, in 2010–2014 (Study III). PC, private (veterinary) clinic; VTH, Veterinary Teaching Hospital (of the University of Helsinki); O, oxacillin; E, erythromycin; C, clindamycin; S, trimetoprim/sulfametoxazol; T, tetracycline; F, fusidic acid; R, enrofloxacin; G, gentamicin; A, amikacin; NT, non-typeable; -, missing value; ST, sequence type; SCCmec, Staphylococcal Cassette Chromosome *mec*. 

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Sma1 PFGE dendrogram with antimicrobial resistance profiles of methicillin-resistant *Staphylococcus pseudintermedius* isolated at the Laboratory of Clinical Microbiology of the Faculty of Veterinary Medicine, University of Helsinki, in 2010–2014 (Study III). PC, private (veterinary) clinic; VTH, Veterinary Teaching Hospital (of the University of Helsinki); O, oxacillin; E, erythromycin; C, clindamycin; S, trimetoprim/sulfametoxazol; T, tetracycline; F, fusidic acid; R, enrofloxacin; G, gentamicin; A, amikacin; NT, non-typeable; -, missing value; ST, sequence type; SCCmec, Staphylococcal Cassette Chromosome *mec*.
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Ascl PFGE dendrogram with antimicrobial resistance profiles of methicillin-resistant *Staphylococcus pseudintermedius* isolated at the Laboratory of Clinical Microbiology of the Faculty of Veterinary Medicine, University of Helsinki, in 2010–2014 (Study III). PC, private (veterinary) clinic; VTH, Veterinary Teaching Hospital (of the University of Helsinki); O, oxacillin; E, erythromycin; C, clindamycin; S, trimetoprim/sulfametoxazol; T, tetracycline; F, fusidic acid; R, enrofloxacin; G, gentamicin; A, amikacin; NT, non-typeable; -, missing value; ST, sequence type; SCCmec, Staphylococcal Cassette Chromosome mec.
Appendix 5

Combinations of result from SCC\textit{mec} M-PCR 1 and 2 that gave a non-typeable outcome (Study III). A–G denote PCR products. A, \textit{mecA} (286 bp); B, \textit{ccrC} (518 bp); C, \textit{ccrA2-ccrB} (937 bp); D, \textit{ccrA4-ccrB4} (1287 bp); E, \textit{mecA-mecI} (1963 bp); F, \textit{mecA-IS431} (804 bp); G, \textit{mecA-IS1272} (2827 bp); n, number of isolates that gave each SCC\textit{mec} result.