Carriage of Methicillin-Resistant *Staphylococcus pseudintermedius* in Small Animal Veterinarians: Indirect Evidence of Zoonotic Transmission

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Impacts

- Five of the 128 veterinarians participating in the study carried methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) clones recently emerged in dogs and cats. This finding indicates that MRSP is not only spreading among pets, as documented by previous studies, but also among veterinarians.
- Methicillin-resistant *Staphylococcus pseudintermedius* was more frequent and more resistant to antibiotics than methicillin-resistant *Staphylococcus aureus*, a multidrug-resistant pathogen of high concern in human medicine.
- Methicillin-resistant *Staphylococcus pseudintermedius* should be regarded as an emerging zoonotic agent as this organism is not part of the human normal microflora and has been reported to cause human infection shortly after its appearance in small animals.

Keywords:
Veterinarians; pet animals; antibiotic resistance; zoonoses; occupational health

Summary

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is increasingly reported in small animals and cases of human infections have already been described despite its recent emergence in veterinary practice. We investigated the prevalence of MRSP and methicillin-resistant *Staphylococcus aureus* (MRSA) among small animal dermatologists attending a national veterinary conference in Italy. Nasal swabs were obtained from 128 veterinarians, seven of which harboured MRSP (*n* = 5; 3.9%) or MRSA (*n* = 2; 1.6%). A follow-up study of two carriers revealed that MRSP persisted for at least 1 month in the nasal cavity. Methicillin-susceptible *S. aureus* (MSSA) was isolated from 32 (25%) conference participants, whereas methicillin-susceptible *S. pseudintermedius* (MSSP) was not detected, suggesting that MRSP may have a particular ability to colonize humans compared to MSSP. All isolates were characterized by *spa* typing. Methicillin-resistant isolates were further typed by antimicrobial susceptibility testing, SCCmec and multi-locus sequence typing. Two lineages previously associated with pets were identified among the five MRSP isolates; the European epidemic clone ST71-SCCmec II-III and ST106-SCCmec IV. One of the two MRSA isolates displayed a genotype (ST22- SCCmecIV) frequently reported in dogs and cats. MRSP isolates were resistant to more antimicrobial agents compared with MRSA isolates and displayed the typical multidrug resistance patterns of MRSP in pets. The 32 MSSA isolates belonged to 20 *spa* types and the most frequent types (t12, t15 and t166) were associated with common *S. aureus* lineages in humans (CC30 and CC45). Although low, the 3.9% MRSP carriage rate found among small animal dermatologists was surprising in consideration of the rare occurrence of *S. pseudintermedius* in humans, the lack of
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**Introduction**

Until few years ago *Staphylococcus intermedius* was considered a canine commensal species and a frequent cause of pyoderma, wound infections, otitis externa and other body tissue infections in dogs and cats (Morris et al., 2006). However, two independent molecular studies by Bannoehr et al. (2007) and Sasaki et al. (2007b) have recently demonstrated that *S. intermedius* is associated with pigeons, whereas the most common staphylococcal species isolated from dogs is *Staphylococcus pseudintermedius*. As proposed by Devriese et al. (2008), in this manuscript, the authors have decided to use the species name *S. pseudintermedius* for referring to past work, in which canine strains were identified as *S. intermedius* by pheno-typic tests.

Methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged in small animals worldwide and represents a serious threat to animal health due to its characteristic multidrug resistance phenotype (Moodley et al., 2009; Perreten et al., 2010). In recent years, the occurrence of MRSP in small animal infections has been increasingly reported in North America (Jones et al., 2007; Hanselman et al., 2008) and Europe (Loeffler et al., 2007; Zubeir et al., 2007; Descloux et al., 2008; van Duijkeren et al., 2008; Schwarz et al., 2008). The epidemic clone circulating in Europe [sequence type (ST) 71] is generally resistant to all antimicrobial agents that are routinely used in small animal practice (Perreten et al., 2010). Similarly to methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistance in MRSP is mediated by acquisition of mecA, which is carried on a mobile genetic element identified as the Staphylococcal Cassette Chromosome mec (SCCmec) (Weese and Duijkeren, 2010). Five different SCCmec types (II-III, III, IV, V and VII) have been detected in MRSP, with SCCmec II-III as the predominant type in ST71 (Perreten et al., 2010).

The most common staphylococci inhabiting the human skin and mucosae are *S. aureus* and coagulase-negative staphylococci. *Staphylococcus pseudintermedius* is a typical commensal of pet animals and its occurrence in humans is mainly limited to individuals having regular contact with dogs and cats, such as small animal veterinarians and pet owners (Talan et al., 1989; Goodacre et al., 1997; Guardabassi et al., 2004). A French study in 1997 (Mahoudeau et al., 1997) indicated that the frequency of *S. pseudintermedius* in clinical specimens from hospitalized patients was very low and accounted for approximately 0.06% (2/3397) coagulase-positive staphylococci. Since the first report of MRSP in Europe in 2007 (Loeffler et al., 2007), cases of human MRSP infection have been reported in Italy (Campanile et al., 2007), in Switzerland (Stegmann et al., 2010) and in the USA (Kempker et al., 2009). Transmission between dogs and staff has been documented at veterinary hospitals in Japan (Sasaki et al., 2007a) and in the Netherlands (van Duijkeren et al., 2008) and a 5% prevalence of MRSP carriage has been recently reported among veterinary dermatology practitioners in the USA (Morris et al., 2010). Pets have also been indicated as potential reservoirs of MRSA (Moodley et al., 2006) and a recent study has shown that veterinary staff and owners of MRSA-infected pets are high risk groups for MRSA carriage despite not having direct hospital links (Loeffler et al., 2010).

The objective of this study was to investigate the prevalence of *S. aureus* and *S. pseudintermedius* in the nasal cavity of small animal dermatologists attending a national veterinary conference in Italy organized by Società Italiana di Dermatologia Veterinaria (SIDEV). Methicillin-resistant and methicillin-susceptible CoPS isolates from the 128 veterinarians participating in the study were genotyped to identify the prevalent genetic lineages of *S. aureus* and *S. pseudintermedius* in the study population.

**Materials and Methods**

**Sampling procedure**

Nasal swabs were collected from 128 small animal dermatologists attending a national veterinary conference in Italy in May 2010. The protocols used were approved by the Danish National Committee on Biomedical Research Ethics (H-KF-2007-0007) and by the scientific committee of the Italian veterinary society organizing the conference. Before collecting the samples, each participant was provided with written instructions on how to collect the sample. A single sterile cotton swab was inserted 1 cm into both nostrils and rotated along the mucosal membrane for 5 s. Participation was on a volunteer basis and all participants provided signed informed consent. Swabs were stored in sterile tubes containing transport medium (Oxoid, Deutschland GmbH, Germany) and delivered to the laboratory (Copenhagen, Denmark) within 5 days for further investigation. MRSP- and MRSA-positive individuals were requested to send nasal swabs for the second detection and the recent appearance of MRSP in Europe. As cases of human MRSP infection have been linked with pets, veterinarians should be aware of this zoonotic risk and proper preventative measures should be taken to avoid MRSP transmission from animal patients.
time 1 month after the initial screening to determine whether carriage was persistent or transient.

Identification and typing of S. aureus and S. pseudintermedius

Swabs were enriched in Mueller Hinton Broth containing 6.5% NaCl for facilitating staphylococcal isolation. After 24 h of incubation at 37°C, 10 µl of the enrichment culture were streaked on 5% calf blood agar and MRSA Brilliance™ agar (Oxoid, Deutschland GmbH, Wesel, Germany) for isolation of total staphylococci and methicillin-resistant staphylococci, respectively. Colonies displaying the typical staphylococcal morphology on blood agar (medium-sized colonies with low convex profile, smooth appearance and entire edge) were subcultured and classified based on haemolytic properties. One colony per plate was isolated unless multiple staphylococcal colonies displaying different colours and/or haemolytic patterns were observed. Suspected MRSP and MRSA colonies displaying light (S. pseudintermedius) or deep (S. aureus) blue colour on MRSA brilliance agar were confirmed as methicillin-resistant by latex PBP2 agglutination test (Oxoid, Basingstoke, England) and meCA PCR (Zhang et al., 2004) and further characterized by SCCmec typing using PCR described by Kondo et al. (2007) and Perreten et al. (2010). The tube coagulase test using rabbit plasma was performed to discriminate between coagulase-positive and coagulase-negative species. Coagulase-positive isolates were further identified by nuc PCR (Sasaki et al., 2010). Staphylococcal protein A (spa) sequence analysis was performed according to the standard protocols for spa typing of S. aureus (Harmsen et al., 2003) or S. pseudintermedius (Perreten et al., 2010). Staphylococcus aureus sequences were submitted to the Ridom spa server database (http://spa.ridom.de/spatypes.shtml, last accessed on 26 July 2010) to determine possible associations with clonal complex (CC). Multi-locus sequence typing (MLST) for S. pseudintermedius was performed according to Bannoehr et al. (2007) on two spa non-typeable MRSP isolates. Allele numbers were determined by comparison with those allele sequences deposited in Genbank. Sequence types (STs) were determined using the key table kindly provided by the curator of the S. pseudintermedius MLST database, Vincent Perreten (vincent.perreten@vbi.unibe.ch). Associations between spa types and STs in S. pseudintermedius were determined according to Perreten et al. (2010). Antimicrobial sensitivity testing by broth microdilution was performed using sensititre COMPAN1F panels (Trek Diagnostics System, East Grinstead, UK) according to the Clinical and Laboratory Standards Institute (2006). The panel included β-lactam (amoxicillin with clavulanic acid, ampicillin, cefazolin, cefovecin, cefoxitin, cefpodoxime, cefotiofur, imipenem, oxacillin with 2% NaCl, ticarcillin with clavulanic acid, ticarcillin and penicillin) and non-β-lactam antibiotics (amikacin, chloramphenicol, gentamicin, clindamycin, doxycycline, enrofloxacin, marbofloxacin, rifampicin and trimethoprim with sulframethoxazole). After 24 h incubation, plates were read using Sensititre Vizion System® (Trek Diagnostics System) and MIC values were interpreted according to the Clinical Laboratory Standards Institute (CLSI) breakpoints for animal and human staphylococci (CLSI, 2006; CLSI, 2008).

Results

A total of 128 veterinarians attending the conference agreed to participate in the study. Based upon isolation on blood agar, 34 participants (26.6%) were positive for S. aureus and five (3.9%) for S. pseudintermedius. Coagulase-negative staphylococci were found in 40.6% (n = 52) of the participants. All positive individuals carried a single species of coagulase-positive species except for one individual who harboured both S. aureus and S. pseudintermedius. One individual was found to carry simultaneously two different S. aureus strains showing different colour and haemolytic patterns on blood agar. The two isolates were confirmed to belong to distinct S. aureus lineages by spa typing (t078 and t338). The 34 S. aureus isolates belonged to 22 spa types (Table 1). The most frequent spa types were t012 (n = 5, CC30), t015 (n = 3, CC45) and t166 (n = 3, CC30).

There was consistency in the recovery of MRSP/MRSA using the two media. Methicillin-resistant staphylococci were isolated on both blood agar and brilliance agar from seven individuals, including five individuals positive for MRSP (3.9% carriage rate) and two positive for MRSA (1.6% carriage rate). Three MRSP isolates belonged to spa type t02 (ST71) and carried SCCmec type II--III. The remaining two MRSP isolates were spa non-typeable and belonged to ST106 and carried SCCmec type IV. The two MRSA isolates displayed spa types t515 (CC22) and t2536 (non-predictable CC) and both carried SCCmec type IV. Two MRSP carriers and one MRSA carrier agreed to participate in the follow-up study and all of them were shown to harbour the same spa types detected in the initial screening.

Different antimicrobials resistance patterns were observed in MRSP and MRSA. All MRSP were resistant to clindamycin, erythromycin and trimethoprim with sulframethoxazole in addition to all β-lactams and susceptible to chloramphenicol, doxycycline and rifampicin. The three MRSP isolates belonging to ST71 exhibited additional resistance to amikacin, gentamicin, enrofloxacin and marbofloxacin. MRSA isolates were resistant to β-lactam antimicrobials and susceptible to all non-β-lactam antimicrobials tested.
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**Table 1. spa type frequency and predicted clonal complex (CC) based on spa typing and multi-locus sequence typing (MLST) of *Staphylococcus aureus* isolated from Italian veterinary dermatology practitioners.**

<table>
<thead>
<tr>
<th>spa type</th>
<th>Frequency (no. positive isolates)</th>
<th>spa CC</th>
<th>MLST CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>t002</td>
<td>2</td>
<td>CC002</td>
<td>CC5</td>
</tr>
<tr>
<td>t010</td>
<td>1</td>
<td>CC002</td>
<td>CC5</td>
</tr>
<tr>
<td>t012</td>
<td>5</td>
<td>CC012</td>
<td>CC30</td>
</tr>
<tr>
<td>t015</td>
<td>3</td>
<td>CC015</td>
<td>CC45</td>
</tr>
<tr>
<td>t021</td>
<td>2</td>
<td>CC021</td>
<td>CC30</td>
</tr>
<tr>
<td>t056</td>
<td>1</td>
<td>CC078</td>
<td>CC25</td>
</tr>
<tr>
<td>t078</td>
<td>1</td>
<td>CC078</td>
<td>CC25</td>
</tr>
<tr>
<td>t084</td>
<td>2</td>
<td>CC084/085</td>
<td>CC15</td>
</tr>
<tr>
<td>t127</td>
<td>1</td>
<td>CC888</td>
<td>CC1</td>
</tr>
<tr>
<td>t166</td>
<td>3</td>
<td>CC166</td>
<td>CC30</td>
</tr>
<tr>
<td>t223</td>
<td>1</td>
<td>CC005</td>
<td>CC22</td>
</tr>
<tr>
<td>t282</td>
<td>1</td>
<td>NP</td>
<td>CCC45</td>
</tr>
<tr>
<td>t289</td>
<td>1</td>
<td>CC084</td>
<td>NP</td>
</tr>
<tr>
<td>t316</td>
<td>1</td>
<td>NP</td>
<td>CC59</td>
</tr>
<tr>
<td>t338</td>
<td>1</td>
<td>NP</td>
<td>CC30</td>
</tr>
<tr>
<td>t458</td>
<td>1</td>
<td>NP</td>
<td>CC97</td>
</tr>
<tr>
<td>t515*</td>
<td>1</td>
<td>NP</td>
<td>CC22</td>
</tr>
<tr>
<td>t5388</td>
<td>2</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>t5516</td>
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<td>NP</td>
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<tr>
<td>t5536*</td>
<td>1</td>
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<tr>
<td>t3856</td>
<td>1</td>
<td>NP</td>
<td>CC425</td>
</tr>
<tr>
<td>t5223</td>
<td>1</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*; NP, not predictable.

*MRSA.*

**Discussion**

Although apparently low, the MRSP carriage rate (3.9%) observed in this study among small animal dermatologists may be regarded as relatively high on the basis of four considerations: (i) *S. pseudintermedius* is not a normal commensal organism in humans (Guardabassi et al., 2004) and a low prevalence (0.75%) of this species has been previously reported in the nasopharyngeal flora of veterinary college staff members (Talan et al., 1989); (ii) as the data above refer to veterinary carriage of total *S. pseudintermedius*, even lower carriage rates would be expected for MRSP, which represents a small fraction of the species population; (iii) MRSP has recently emerged and the first report in dogs in Europe was dated 2007 (Loeffler et al., 2007); and (iv) the MRSP carriage rate was higher than for MRSA, which is the methicillin-resistant staphylococcal species traditionally associated with humans. These data provide indirect evidence of zoonotic transmission of MRSP. Small animal dermatologists may have an increased risk for acquisition of MRSP because they are regularly exposed to small animals with skin and soft tissue infections. Our data are consistent with the results of a recent study in the USA, in which MRSP and MRSA carriage rates of 5.3% and 3.5%, respectively, were reported in a sample of 171 veterinary dermatologists (Morris et al., 2010). In addition to generating prevalence data relative to a different geographical region, our work provides preliminary evidence of potential long-term carriage of MRSP in humans and epidemiological information on the carriage of methicillin-susceptible *S. pseudintermedius* (MSSP) and *S. aureus* (MSSA), on the genetic background of coagulase-positive staphylococci isolated from veterinary staff.

The finding that veterinarians carried MRSP but not MSSP is particularly interesting as it suggests that MRSP may have a particular predisposition to human colonization compared to MSSP. Although the prevalence of MRSP in pets has rapidly increased over the last 3 years, MSSP is still largely predominant in the *S. pseudintermedius* population and it is reasonable to assume that veterinarians are more exposed to MSSP than to MRSP during their work. Therefore, a significantly higher MSSP carriage rate would be expected if MRSP and MSSP had equal ability to colonize humans. The recovery of MRSP but not MSSP from the nasal cavity of veterinarians might be associated with a particular ability to adapt to the human host by the *S. pseudintermedius* lineages that have acquired methicillin-resistance such as ST71 and ST106. This hypothesis is further supported by the fact that MRSP carriage was persistent over a period of 1 month in the two veterinarians who agreed to participate in the follow-up study. To our knowledge, this study is the first study investigating occurrence of both MRSP and MSSP among veterinarians and suggesting prolonged human carriage of MRSP. Although this phenomenon is suggestive of true colonization, ‘re-infection’ cannot be excluded as veterinarians have frequent contact with infected pets and many of them have either a dog or a cat at home.

The MRSP strains isolated from Italian veterinarians corresponded to the major MRSP clones recently emerged in the pet population in Europe (Perreten et al., 2010). The rapid spread of MRSP ST71 is of particular concern as this clone tends to be resistant to all antimicrobial agents that are routinely used in small animal practice, including the last choice drugs such as aminoglycosides and fluoroquinolones. This characteristic multidrug resistance pattern was also observed in the three human MRSP ST71 isolates described in this study. Notably, all five MRSP isolates were resistant to more antimicrobial agents in comparison to MRSA, which is generally regarded as one of the most resistant bacteria in human medicine. The first human case of infection with MRSP ST71 has recently been reported in Switzerland in a patient with a history of recurrent rhinosinusitis and
three surgical interventions (Stegmann et al., 2010). This person owned a dog with severe clinical problems but the dog was killed before samples could be taken to determine whether it was a MRSP carrier. In a previous study in the USA (Kemper et al., 2009), an MRSP infection was reported in a woman affected by sinusitis and the strain was shown to be epidemiologically related to that carried by her dog. Before the current typing scheme for *S. pseudintermedius* was established, an MRSP strain isolated from a bacteremic patient in Italy was genetically characterized (Campanile et al., 2007) and the SCCmec element carried by this clinical strain does not corresponded to the characteristic structure of SCCmec type II-III associated with ST71 (Perreten et al., 2010). Altogether it appears that MRSP and in particular, the European epidemic clone ST71 is rapidly spreading in the human population and may have an increased pathogenic potential compared with MSSP. It should be noted that MRSP can be misidentified as MRSA in hospital clinical laboratories due to close phenotypic similarity between the two species (Pottumarthy et al., 2004). Consequently, the real incidence of human infections associated with this emerging zoonotic agent may presently be underestimated.

Italy is one of the European countries with high prevalence of MRSA in human *S. aureus* infections. According to the European Antimicrobial Resistance Surveillance System (EARSS) data for 2008 (http://www.rivm.nl/earss/database/, accessed on 6 September 2010), 35.5% of *S. aureus* isolates from bacteremia in this country are MRSA. The prevalence (1.6%) of MRSA carriage in this study is comparable to those reported among small animal practitioners attending veterinary conferences in Denmark (3.0%) (Moodley et al., 2008) and in North America (3.3%) (Hanselman et al., 2009). This prevalence is higher than the expected carriage rate among healthy individuals in the community (0.1–0.8%) (Gzanelli et al., 2002; Wertheim et al., 2004; Kuehnert et al., 2006). According to a study conducted in central Italy in 2002 (Gzanelli et al., 2002), the rates of nasal MRSA and MSSA carriage in the community are 0.1% (1/812) and 30.5% (248/812), respectively. Interestingly, one of the two MRSA isolated in this study displayed a *spa* type (t515) that has been reported among clinical isolates in Italy (Borghi et al., 2010; Monaco et al., 2010) and belongs to CC22, a genetic lineage that has previously been associated with small animal veterinarians in the UK and Ireland (Moodley et al., 2006).

The study also provides information about the distribution of *S. aureus* lineages circulating in the community in Italy. The prevalence of MSSA carriage (25%) was normal in relation to the expected percentage of *S. aureus* carriers in the general population in Italy (Gzanelli et al., 2002). It is generally assumed that approximately 20% of people are permanent carriers and 30% are transiently colonized during the course of their life (Eriksen et al., 1995; Hu et al., 1995; Wertheim et al., 2005). The most prevalent MSSA *spa* types corresponded to CC30 (*n* = 11) and CC45 (*n* = 4), which are two among the most widespread MSSA lineages in the community. Between 1961 and 2004, data from six continents including Europe suggest that CC30 is the major *S. aureus* CC circulating in the human population (Feil et al., 2003; Chambers and DeLeo, 2009).

**Conclusion**

The 3.9% MRSP carriage rate observed in this sample of small animal dermatologists in Italy was surprising in consideration of the rare occurrence of *S. pseudintermedius* in humans, the lack of MSSP detection and the recent appearance of MRSP in Europe. The results indicate that this specific occupational group may be at a higher risk for MRSP carriage. MRSP should be regarded as an emerging zoonotic agent as this organism is not part of the normal microflora and pet-associated infections have already been reported in people despite its recent emergence in small animals. Infection control measures are required to minimize the spread of this multidrug-resistant pathogen from healthy animal carriers and patients to veterinarians or owners. Clinical laboratories should be aware of the emergence of this new zoonotic bacterium in order to avoid possible MRSP misidentification as MRSA.

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