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2017-04


http://hdl.handle.net/10138/190462
https://doi.org/10.1093/jac/dkw559

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Antimicrobial resistance in Staphylococcus pseudintermedius and the molecular epidemiology of methicillin-resistant S. pseudintermedius in small animals in Finland

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Received 17 August 2016; returned 9 October 2016; revised 3 November 2016; accepted 30 November 2016

Objectives: To investigate antimicrobial susceptibility in Staphylococcus pseudintermedius and the occurrence of methicillin-resistant S. pseudintermedius (MRSP), to explore the molecular structure of the MRSP population and to analyse risk factors for MRSP.

Methods: Susceptibility data for clinical S. pseudintermedius isolates in 2011–15 were analysed using WHONET. All MRSP isolates in 2010–14 (n = 362) were typed using PFGE. Representative isolates (n = 87) of clusters were analysed using MLST and staphylococcal cassette chromosome mec (SCCmec) typing. Risk factors were analysed using logistic regression.

Results: Of the clinical S. pseudintermedius (n = 1958; 98% from dogs), 14% were MRSP. Resistance to other antimicrobials varied between 12% and 39%. No trends were observed over time. Among clinical specimens (from infection sites) and screening specimens (from potential carriers), respectively, 2.5% (267/10 813) and 9% (211/2434) revealed MRSP. MLST revealed 42 different STs, including 19 new ones. Clonal complexes 71, 45 and 258 were the most common, but the MRSP population diversified over the years. A clinical S. pseudintermedius isolate was more likely to be MRSP if the patient was on antimicrobials at the time of sampling or was male. The presence of MRSP in screening specimens was more likely if the patient was on multiple antimicrobials at the time of sampling. Specimens from private clinics (versus the Veterinary Teaching Hospital of the University of Helsinki) had a higher likelihood of MRSP in both analyses.

Conclusions: Resistance to antimicrobials among S. pseudintermedius in Finland is high, emphasizing the importance of infection control measures and susceptibility testing prior to therapy. The diverse MRSP population indicates non-clonal spread.

Introduction

Staphylococcus pseudintermedius is a part of the normal microbiota of dogs and cats.1,2 This opportunistic bacterium can cause a wide range of infections, ranging from pyoderma and surgical wound infections to deep infections such as osteomyelitis.3–6 Over the past decade, methicillin-resistant S. pseudintermedius (MRSP) has become a worldwide problem in small animal veterinary medicine.7,8 Ever fewer effective antimicrobials are available for treatment, as MRSP isolates are commonly MDR.8–10

The epidemiology and molecular characteristics of MRSP in neighbouring countries, such as Sweden and Norway, have already been explored.11–13 There is evidence that the clonal structure of MRSP is changing in some countries.13,14 In Finland, a large veterinary hospital outbreak in 2010–11 was caused by the predominant European MRSP clone, ST71.15 The study also identified ST45 as causing a smaller cluster. These two main clones were additionally identified among Finnish guide dogs, but the presence of other unrelated STs indicated diversity in the MRSP population.16 However, no study has extensively explored the epidemiology of MRSP in Finland. The goal of this study was to investigate the epidemiology of S. pseudintermedius in Finland during 2010–15 by (i) reporting the antimicrobial susceptibility time trends of clinical isolates in 2011–15, (ii) exploring the molecular structure of the MRSP population in 2010–14, (iii) describing yearly MRSP proportions in clinical
specimens and screening specimens from potential carriers and (iv) determining risk factors for MRSP among screening specimens and for a clinical S. pseudintermedius being MRSP.

**Methods**

**Study setting**

The investigation was carried out at the Clinical Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Helsinki. In 2011–15, the laboratory processed 19 249 microbiological specimens.

**Data collection**

For analysis of antimicrobial susceptibility, results for all isolates identified as S. pseudintermedius between June 2011 and the end of 2015 were compiled from the laboratory information system (LIS) (Provet Net, Finnish Net Solutions, Finland). In addition, patient information data (species, sex, specimen type, presence of antimicrobial therapy at the time of sampling, and submitting clinic) were collected. Antimicrobials tested in the basic panel included clindamycin, erythromycin, fusidic acid, oxacillin, trimethoprim/sulfamethoxazole and tetracycline. Comparable data for the extended panel (aminoglycoside, chloramphenicol, doxycycline, enrofloxacin and gentamicin) were only available for 2015. Susceptibility testing was performed by disc diffusion according to CLSI standards.19,20

**Molecular methods**

Suspected MRSP isolates, based on resistance to oxacillin,26 were confirmed to carry the mecA gene by PCR.16 All confirmed isolates were stored at −80°C until further studied. To detect clonal clusters, all MRSP isolates stored in 2010–14 were digested by SmaI macrorestriction (New England Biolabs, USA) and separated by PFGE21 with modifications.15 Isolates that were non-typeable by SmaI were digested using AscI (New England Biolabs, USA). PFGE clusters were illustrated with GelComparr II (v. 6.5; Applied Maths NV, Belgium) by UPGMA-based analysis using the Dice similarity coefficient with an 80% similarity cut-off; optimization and position tolerance were set at 1.2% and 1.3%, respectively. At least one isolate from each PFGE cluster was selected for further characterization by MLST and staphylococcal cassette chromosome mec (SCCmec) typing,22,23 with modifications.16 For some isolates, the MLST sequencing result for the tuf gene was poor with published primers.22 Therefore, the primers were modified as follows: tuf 19F, 5′-GTCCAATGCCACAAACTCG-3′; and tuf 19R, 5′-CCACGTTCAGCTGTACCTA-3′. MLST results for isolates were extrapolated to all members of the PFGE cluster. Isolates for which data had previously been published were included in the study.15,16

**Data analysis**

Susceptibility data for clinical S. pseudintermedius isolates (screening specimens excluded) were analysed using WHONET (v. 5.6, WHO). Non-susceptibility percentages, including resistant and intermediate isolates, with 95% CIs, were calculated and presented separately for MRSP, methicillin-susceptible S. pseudintermedius (MSSP) and all S. pseudintermedius isolates. CLSI breakpoints were used.19,20 except for fusidic acid, for which a non-susceptibility breakpoint of ≤23 μg/mL was used.24 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 χ² test based on the WHONET output. P values <0.05 were considered statistically significant. Analyses were performed with the SAS® System for Windows (v. 9.3, USA). To calculate the number of MDR isolates (resistance to at least three antimicrobial classes), macrolide and lincosamide resistance was pooled due to common MLSb resistance (macrolide, lincosamide and streptogramin B).25

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. Patients in the latter populations have previously 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).15 Screening is targeted at these patients.

To compare the genetic relatedness of STs, the allele sequences for each ST were added back-to-back (ack, cmp60, fdh, pta, purA, sar, tuf). The resulting sequences were aligned and a phylogenetic tree was inferred by the Bayesian Markov Chain Monte Carlo (MCMC) method implemented in BEAST (v. 1.7.2).26 Each run was continued until the effective sample size (ESS) was > 200. Posterior probabilities were calculated with a 10% burn-in and values >0.7 were considered significant. Results were visualized in FigTree (v. 1.4.0). Additionally, gbeBURST (v 1.2.1) software was used for population structure analysis of STs.27 Analysis was conducted at double- and triple-locus variant levels. Single- and double-locus variants of previously described clonal complexes (CCs) were assigned to that CC.14 The number of isolates per ST or CC per year was calculated based on the specimen collection date.

For analysis of predictors for MRSP, data were analysed separately 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 Veterinary Teaching Hospital of the University of Helsinki (VTH) in 2010–11 were likely to skew the results, data for this period were omitted.15 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. ORs with 95% CI 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. P values ≤0.05 were considered statistically significant in the final model. Analyses were performed using SPSS v. 24 (IBM Inc.).

**Results**

**Clinical S. pseudintermedius isolates**

Results were available from a total of 1958 clinical S. pseudintermedius isolates. Of these, 1671 (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 11 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 recovered 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 further testing.

**Antimicrobial susceptibility and occurrence of MRSP**

Out of the 1958 clinical S. pseudintermedius isolates, the overall proportion of oxacillin (methicillin) resistance was 14% (n = 266). Non-susceptibility data for all tested antimicrobials for MRSP, MSSP and overall are presented in Table 1. The proportion of non-susceptible isolates per year varied only slightly for each antimicrobial and no statistically significant trends were detected (Figure 1).
Table 1. Antimicrobial non-susceptibility among clinical S. pseudintermedius isolates from mid-2011 to the end of 2015 in Finland

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MRSP % non-susceptible (95% CI)</th>
<th>MRSP number of isolates</th>
<th>MSSP % non-susceptible (95% CI)</th>
<th>MSSP number of isolates</th>
<th>All % non-susceptible (95% CI)</th>
<th>All number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic panel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>clindamycin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.7 (80.8–89.6)</td>
<td>265</td>
<td>22.2 (20.2–24.3)</td>
<td>1678</td>
<td>30.8 (28.8–32.9)</td>
<td>1947</td>
</tr>
<tr>
<td>erythromycin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.7 (80.8–89.6)</td>
<td>266</td>
<td>21.8 (19.9–23.9)</td>
<td>1678</td>
<td>30.5 (28.5–32.6)</td>
<td>1949</td>
</tr>
<tr>
<td>fusidic acid</td>
<td>24.5 (19.5–30.2)</td>
<td>265</td>
<td>24.3 (22.3–26.4)</td>
<td>1677</td>
<td>24.4 (22.5–26.4)</td>
<td>1946</td>
</tr>
<tr>
<td>oxacillin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100 (98.2–100)</td>
<td>266</td>
<td>0.0 (0.0–0.3)</td>
<td>1682</td>
<td>13.7 (12.2–15.3)</td>
<td>1948</td>
</tr>
<tr>
<td>tetracycline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74 (68.2–79.1)</td>
<td>265</td>
<td>33.3 (31.1–35.6)</td>
<td>1675</td>
<td>38.8 (36.6–41.0)</td>
<td>1944</td>
</tr>
<tr>
<td>trimethoprim/sulfamethoxazole&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.7 (41.6–53.9)</td>
<td>266</td>
<td>6.1 (5.0–7.4)</td>
<td>1680</td>
<td>11.8 (10.4–13.3)</td>
<td>1951</td>
</tr>
<tr>
<td><strong>Extended panel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amikacin</td>
<td>0 (0.0–2.1)</td>
<td>219</td>
<td>0 (0.0–0.9)</td>
<td>547</td>
<td>0 (0.0–1.2)</td>
<td>393</td>
</tr>
<tr>
<td>chloramphenicol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.9 (29.5–65.0)</td>
<td>32</td>
<td>15 (10.6–20.8)</td>
<td>207</td>
<td>18.4 (13.7–24.2)</td>
<td>228</td>
</tr>
<tr>
<td>doxycycline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.8 (18.6–41.4)</td>
<td>66</td>
<td>4.7 (2.8–7.7)</td>
<td>342</td>
<td>6.1 (4.0–9.1)</td>
<td>392</td>
</tr>
<tr>
<td>enrofloxacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.4 (44.0–56.8)</td>
<td>248</td>
<td>2.6 (1.7–4.0)</td>
<td>793</td>
<td>7.3 (5.0–10.4)</td>
<td>395</td>
</tr>
<tr>
<td>gentamicin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.8 (38.5–51.2)</td>
<td>248</td>
<td>2.5 (1.6–3.9)</td>
<td>795</td>
<td>6.6 (4.4–9.6)</td>
<td>395</td>
</tr>
</tbody>
</table>

<sup>a</sup>Statistically significant (<i>P</i> < 0.001) difference between MRSP and MSSP.

<sup>b</sup>Consistent data only available for 2015, as the extended panel was only investigated for MRSP or otherwise MDR isolates prior to this.

**Figure 1.** Non-susceptibility percentages by year with 95% CIs for clinical S. pseudintermedius isolates in Finland from mid-2011 to the end of 2015. Cochran–Armitage trend test <i>P</i> 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 (trimethoprim/sulfamethoxazole) and 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. The proportion of MRSP among clinical and screening specimens by year is presented in Table 2.

### Molecular characteristics of MRSP

Out of the 362 MRSP isolates (197 from clinical specimens, 165 from screening specimens), 279 were typeable using SmaI macrorestriction. These clustered into 19 (A–S) different clusters with four or more isolates (Figure S1, available as Supplementary data at JAC Online). Eighty-three isolates could only be typed by AscI macrorestriction and formed two clusters (T and U) and one singleton in PFGE analysis (Figure S2). 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 (STs 621 and 625 to 642). 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 2). All identified STs and their clonal and genetic relatedness are presented in Figure 3(a and b). Six STs grouped together with previously described CCs (CC45 and CC258) (Figure 3a). STs in the CC258 group were scattered in the sequence comparison tree, while STs in the CC45 group were more alike (Figure 3b).

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 (Figure S3). The number of isolates found to carry each SCCmec, as well as the STs in which these were found, are presented in Table 3.

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**Table 2. Proportion of MRSP per year among clinical and screening specimens from dogs and cats in Finland in 2011–15**

<table>
<thead>
<tr>
<th>Year</th>
<th>Clinical specimens</th>
<th></th>
<th>Screening specimens</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dogs</td>
<td>cats</td>
<td>dogs</td>
<td>cats</td>
</tr>
<tr>
<td></td>
<td>MRSP %</td>
<td>number of specimens</td>
<td>MRSP %</td>
<td>number of specimens</td>
</tr>
<tr>
<td>2011 June–a</td>
<td>3.6</td>
<td>933</td>
<td>2.3</td>
<td>130</td>
</tr>
<tr>
<td>2012</td>
<td>3.3</td>
<td>2076</td>
<td>0.3</td>
<td>358</td>
</tr>
<tr>
<td>2013</td>
<td>2.6</td>
<td>2104</td>
<td>0.0</td>
<td>368</td>
</tr>
<tr>
<td>2014</td>
<td>2.6</td>
<td>1963</td>
<td>0.3</td>
<td>382</td>
</tr>
<tr>
<td>2015</td>
<td>2.5</td>
<td>2098</td>
<td>0.2</td>
<td>401</td>
</tr>
<tr>
<td>Total</td>
<td>2.8</td>
<td>9174</td>
<td>0.4</td>
<td>1639</td>
</tr>
</tbody>
</table>

aThe MRSP outbreak at the VTH was still ongoing in 2011.
Predictors of MRSP

According to the results of multivariable logistic regression analysis, a clinical S. pseudintermedius isolate was more likely to be MRSP if the specimen was from a private clinic (14% of isolates versus 9% of isolates from the VTH), the patient was male or the patient was on any antimicrobials. Among patients who had received antimicrobials, those receiving β-lactams had higher odds of MRSP (Table 4). For screening specimens, the detection of MRSP was more likely if the patient was being treated with multiple systemic antimicrobials (most commonly a combination of

Figure 3. Genetic relationship of MRSP in Finland (2010–14). (a) goeBURST analysis conducted at the double-locus variant level, with triple-locus variants (connected with grey dashed lines) added to show further relatedness. Line numbers and shading indicate the number of differing loci between STs. Grey areas highlight CCs. Grey boxes with black text, group founder; black boxes with white text, sub-group founder; white boxes with black text, common node; grey 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. Grey shades indicate CC groups as assigned by goeBURST analysis. *CC founder; †single-locus variant; ‡double-locus variant.
ampicillin and enrofloxacin) or if the specimen was from a private clinic (Table 5).

**Discussion**

The proportion of MRSP among clinical *S. pseudintermedius* isolates was fairly high, being nearly 14%. The high percentage (17%) in 2011 was influenced by the MRSP outbreak at the VTH.\(^{15}\) Interestingly, the proportions of MRSP among isolates from private clinics and the VTH were significantly different. This difference may be due to dissimilar patient populations. While the VTH is a national referral veterinary hospital, it also treats first-opinion patients. Numerous clinical specimens originated from private clinics that are treating many dermatological patients, a group at risk of MRSP due to frequent antimicrobial therapy and veterinary visits.\(^{15,16,29–31}\) A threshold to obtain bacteriological specimens early in the infection process is probably higher among private clinic veterinarians. The VTH has had a strict policy of obtaining specimens in all cases of suspected bacterial infection since the MRSP outbreak in 2010–11.\(^{15}\) It is also likely that screening criteria for risk patients are wider at the VTH compared with private clinics, which, in our experience, mainly screen dermatological patients. As the specimens from private clinics may predominantly originate from chronic cases, a higher rate of resistant bacteria would be expected. A comparable difference in patient populations was the likely explanation for unequal methicillin resistance rates between two laboratories in the UK.\(^{32}\) At the microbiology laboratory of the Royal Veterinary College (RVC), 14% of *Staphylococcus intermedius* group isolates in 2006–12 were oxacillin resistant, while oxacillin resistance in another laboratory was only 1%. This difference was attributed to the high resistance among referral animals of the RVC.\(^{32}\)

Non-susceptibility to antimicrobials was significantly higher among MRSP isolates than MSSP isolates, except for fusidic acid, for which non-susceptibility was around 24% in both. Similar findings apply to MRSA and MSSA.\(^{33}\) 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),\(^{13}\) which is similar to our results. In Norway, nearly half of *S. pseudintermedius* isolates investigated were fusidic acid resistant (MIC >1 mg/L).\(^{26}\)

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**Table 3.** Number of MRSP isolates with each identified SCCmec and the STs associated with them

<table>
<thead>
<tr>
<th>SCCmec</th>
<th>Number of isolates</th>
<th>ST(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>

**Table 4.** Risk factors associated with the discovery of MRSP among canine clinical *S. pseudintermedius* isolates taken during 2012–15

<table>
<thead>
<tr>
<th>MRSP (n = 227)</th>
<th>MSSP (n = 1501)</th>
<th>Univariable logistic regression</th>
<th>Multivariable logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(^a)</td>
<td>%(^a)</td>
<td>n(^a)</td>
</tr>
<tr>
<td>Private clinic versus university teaching hospital</td>
<td>192</td>
<td>84.6</td>
<td>1140</td>
</tr>
<tr>
<td>Gender: male versus female</td>
<td>150</td>
<td>67.6</td>
<td>761</td>
</tr>
<tr>
<td>Deep lesion specimen</td>
<td>31</td>
<td>13.7</td>
<td>234</td>
</tr>
<tr>
<td>Superficial lesion specimen</td>
<td>173</td>
<td>76.2</td>
<td>1147</td>
</tr>
<tr>
<td>Urine specimen</td>
<td>8</td>
<td>3.5</td>
<td>81</td>
</tr>
<tr>
<td>Other specimens</td>
<td>15</td>
<td>6.6</td>
<td>39</td>
</tr>
<tr>
<td>Antimicrobial treatment during sampling</td>
<td>84</td>
<td>40.2</td>
<td>264</td>
</tr>
<tr>
<td>Antimicrobial groups(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systemic β-lactams</td>
<td>60</td>
<td>71.4</td>
<td>150</td>
</tr>
<tr>
<td>multiple systemic antimicrobials</td>
<td>1</td>
<td>1.2</td>
<td>6</td>
</tr>
<tr>
<td>other systemic antimicrobials</td>
<td>15</td>
<td>17.9</td>
<td>63</td>
</tr>
<tr>
<td>topical antimicrobials</td>
<td>8</td>
<td>9.5</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^a\)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 example, antimicrobial treatment data were available from 209 dogs among MRSP cases (84/0.402).

\(^b\)Variables eliminated from the final models.

\(^c\)Antimicrobial group variables were analysed separately for patients that had received antimicrobials.

### Additional Notes

- P values in bold indicate variables included in multivariate analysis (P < 0.2).
- 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 example, antimicrobial treatment data were available from 209 dogs among MRSP cases (84/0.402).
- Variables eliminated from the final models.
- Antimicrobial group variables were analysed separately for patients that had received antimicrobials.
Epidemiology of MRSP in small animals in Finland

On the other hand, an Italian study did not find fusidic acid resistance in S. intermedius. A recent report from Sweden details resistance among S. pseudintermedius collected from skin lesions. 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. Due to the high resistance for 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. This is also stated in the newly published national guidelines on the use of antimicrobials and infection control policies in these countries. 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. Note, however, that in 2010–11 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 MRSP proportion was observed. A Swedish study (isolates from 2008 to mid-2010) showed a fairly homogeneous MRSP population, with 96% (216/226) of the isolates representing the predominant European lineage, ST71. This clone was also the most common ST in Finland in 2010–11, but has diminished since (Figure 2). Similarly to our data, a change in the MRSP population has also been observed in Sweden after 2010. There, 20 out of 58 MRSP isolates (34%) were ST258 in 2015. CC258 was the third most common CC in Sweden after 2010. There, 20 out of 58 MRSP isolates (34%) were ST258 in 2015. Further research is required to determine the value of antimicrobial therapy in patients when underlying conditions have been controlled.

### Table 5. Risk factors associated with the discovery of MRSP among canine screening specimens taken during 2012–15

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>MRSP positive (n = 173)</th>
<th>MRSP negative (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>unadjusted OR (95% CI)</td>
<td>univariate P</td>
</tr>
<tr>
<td>Private clinic versus university teaching hospital</td>
<td>67</td>
<td>38.7</td>
<td>1.45 (1.05–2.00)</td>
<td>0.024</td>
</tr>
<tr>
<td>Gender: male versus female</td>
<td>92</td>
<td>54.4</td>
<td>1.19 (0.86–1.63)</td>
<td>0.295</td>
</tr>
<tr>
<td>Antimicrobial treatment during sampling</td>
<td>45</td>
<td>29.4</td>
<td>1.04 (0.73–1.50)</td>
<td>0.815</td>
</tr>
<tr>
<td>Systemic β-lactams</td>
<td>20</td>
<td>13.1</td>
<td>0.92 (0.57–1.51)</td>
<td>0.751</td>
</tr>
<tr>
<td>Multiple systemic antimicrobials</td>
<td>10</td>
<td>6.5</td>
<td>1.88 (0.94–3.77)</td>
<td>0.074</td>
</tr>
<tr>
<td>Other systemic antimicrobials</td>
<td>7</td>
<td>4.0</td>
<td>0.63 (0.29–1.37)</td>
<td>0.243</td>
</tr>
<tr>
<td>Topical antimicrobials</td>
<td>6</td>
<td>3.9</td>
<td>1.38 (0.58–3.28)</td>
<td>0.470</td>
</tr>
</tbody>
</table>

P values in bold indicate variables included in multivariate analysis (P ≤ 0.2).
a % 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 example, antimicrobial treatment data were available from 153 dogs among MRSP cases (45/0.294). Antimicrobial treatment in general was not significant in the univariable analysis and antimicrobial groups were therefore included in the model.
our study and was the largest clone in Norway in a recent study.\textsuperscript{12} Additionally, CC258 has recently been reported as a major CC in both the Netherlands\textsuperscript{14} and Denmark.\textsuperscript{45} These studies also reported a marked diversity in the MRSP population in recent years.\textsuperscript{14,45} It appears that ST71 has lost its sustainability and CC258 is only filling the gap as a more successful lineage. ST71 carries SCC\textsuperscript{mec} II–III, which has only been found in related STs and ST354.\textsuperscript{45,46} The immobility of this element may be an underlying reason for the demise of the clone. In contrast, our data indicate that SCC\textsuperscript{mec} 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 SCC\textsuperscript{mec} 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 SCC\textsuperscript{mec} 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 annual share of the MRSP isolates of \textasciitilde 20% in 2011–14. Representatives of this CC have been detected at least in Sweden,\textsuperscript{37} the Netherlands\textsuperscript{14} and in Israel and Thailand.\textsuperscript{48,49} but have been rather rare in reports from Europe.\textsuperscript{14,47} Representatives of ST45 are typically non-typeable by Smal PFGE,\textsuperscript{48} 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 (Figure S2). 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 (six nucleotide differences in one locus), while ST638, a double-locus variant of ST258, was deemed nearly identical to ST258 (four nucleotide differences in two loci) in the maximum likelihood tree (Figure 3a and b). Performing eBURST analysis of MLST data has become common when analysing the clonality of MRSP. However, it does seem 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 (3–26). It could be beneficial to determine genetic relatedness based on the actual sequences, as was done by Kjellman et al.,\textsuperscript{12} rather than by comparing combinations of allele numbers. It is, however, likely that WGS will replace eBURST analysis once its costs decrease.

There are some limitations in this study. The 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. In addition, information on risk factors, such as antimicrobial therapy, is prone to reporting bias, as this information is more likely to be reported if the animal is receiving treatment. This is unlikely to have impacted our results, as the minimum data coverage for risk factors was 88%. In addition, susceptibility data may contain more than one isolate from the same animal, which may have caused bias in the data. The bias caused by this 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. Furthermore, PFGE was used as a screening method to find candidates for MSLT and SCC\textsuperscript{mec} 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.

Conclusions
To conclude, the high resistance to methicillin and other antimicrobials among \textit{S. pseudintermedius} is of concern. Currently, MRSP in Finland may be spreading via gene transfer rather than clonally, which makes it more difficult to predict resistance patterns in \textit{S. pseudintermedius} and prevent MRSP. Additionally, routine screening of cats for MRSP is not necessary due to the low occurrence, even among risk patients. We emphasize the need to obtain a bacteriological specimen whenever antimicrobial therapy is indicated. The importance of infection control in veterinary premises should also be stressed as a means of preventing the spread of resistance. The use of non-antimicrobial therapy should be considered whenever feasible.

Acknowledgements
Jouni Jumnila is acknowledged for his expertise with statistical analyses. We thank Marja Matikka and Gina Meder for their excellent technical assistance and we thank Ray Siddall for revising the language of the manuscript. We thank the Alfred Kordelin Foundation for their generous grant to T. G. and the Helvi Knuuttila Foundation for a grant for material expenses that made the molecular typing possible.

Funding
This work was supported by the Alfred Kordelin Foundation (grant number 140171 to T. G.) and the Helvi Knuuttila Foundation (material grant).

Transparency declarations
None to declare.

Supplementary data
Figures S1 to S3 are available as Supplementary data at JAC Online.

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