Genotypic relatedness and characterization of *Staphylococcus pseudintermedius* associated with post-operative surgical infections in dogs

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*Staphylococcus pseudintermedius* is a commensal organism of dogs that can also be implicated in surgical site infections (SSIs) in dogs. Particularly with the recent emergence and spread of the ST71-t02-SCCmeccII–III multidrug-resistant *S. pseudintermedius* clonal lineage (MDRSP), it is important to understand the clonal diversity of *S. pseudintermedius* in SSIs in dogs. The study reported here investigated the genotypic relatedness of 124 *S. pseudintermedius* isolates from the surgical wounds of 90 dogs admitted to a referral practice in the UK. This study also aimed to understand whether MDRSP is better adapted to survival and persistence in different environments compared with other *S. pseudintermedius*.

Whilst no individual *S. pseudintermedius* clonal type was primarily responsible for *S. pseudintermedius*-associated SSIs in dogs, we found that MDRSP was the most represented clonal type among the isolates studied. However, we observed no difference in the level of biofilm production, susceptibility to biocides or carriage of specific virulence determinants between MDRSP and other *S. pseudintermedius* isolates studied. Interestingly, in the competitive fitness study, MDRSP did not outcompete any member of the other *S. pseudintermedius* isolates studied in each environment. Our data suggest that the determinants that promote *S. pseudintermedius*-associated SSIs in dogs are distributed among *S. pseudintermedius* as a species and are not restricted to a few clonal types. They also provide evidence to support the suggestion that MDRSP is not better adapted to survival or persistence in different environments and is no more virulent than other *S. pseudintermedius* isolates.

**INTRODUCTION**

*Staphylococcus pseudintermedius* is a Gram-positive, facultative, anaerobic coccus that naturally colonizes the skin, coat, nose, mouth and anus of dogs (van Duijkeren *et al.*, 2011). *S. pseudintermedius* is also an opportunistic pathogen in dogs where it is the bacterium primarily implicated in surgical infections, pyoderma, otitis externa, endometritis and other suppurative conditions (Quinn *et al.*, 2011).

**Abbreviations:** MBC, minimum bactericidal concentration; MDRSP, multidrug-resistant *S. pseudintermedius* clonal lineage; PVL, Panton–Valentine leukocidin; SIET, *Staphylococcus intermedius* exfoliative toxin; TSB, tryptic soy broth.

One supplementary figure is available with the online Supplementary Material.
staphylococcal enterotoxins and biofilm production factors have been identified (Bannoehr et al., 2012; Fitzgerald, 2009; Sasaki et al., 2007; van Duijkeren et al., 2011). The recent emergence and spread of the multidrug-resistant clonal lineage is particularly significant as it may be associated with an overall increase in the length of hospitalization and a further increase in healthcare costs, mortality and morbidity (Cosgrove, 2006; de Kraker et al., 2011). As a consequence, the multidrug-resistant clonal type is considered a major therapeutic challenge and an important problem in veterinary medicine (de Lucia et al., 2011; Moodley et al., 2009). Therefore, it is important to understand whether this clonal lineage is better adapted to survival and persistence in different environments than other S. pseudintermedius strains. This understanding is important as it may inform potential strategies for its control.

The work presented here aimed to determine the species diversity among S. pseudintermedius implicated in S. pseudintermedius-associated surgical site infections (SSIs). We also aimed to understand whether the ST71-t02-SCCmecII–III multidrug-resistant S. pseudintermedius clonal lineage (MDRSP) clonal type is better adapted to survival and persistence in different environments compared with other S. pseudintermedius strains.

METHODS

Bacterial isolates studied. To determine the genotypic relatedness of S. pseudintermedius associated with surgical infections following orthopaedic intervention, 124 S. pseudintermedius isolates were obtained from the culture collection of an orthopaedic referral veterinary practice based in the UK. These S. pseudintermedius isolates were cultured over a 2-year period from the infected surgical wounds of 90 dogs that were referred to the practice.

Molecular identification of S. pseudintermedius. Identification of the S. pseudintermedius isolates used in the study was performed by MboI restriction digests of the pta gene fragment, as described previously (Bannoehr et al., 2009).

Comparative analysis of multidrug-resistant and susceptible S. pseudintermedius isolates. A subset of the 124 S. pseudintermedius isolates studied (13 multidrug-resistant and 13 susceptible isolates) was comparatively analysed. Multidrug resistance was defined by resistance to antibiotics belonging to three or more antibiotic classes. These antibiotics included: amikacin, amoxicillin-clavulanic acid, ampicillin, ciprofloxacin, cephalaxin, gentamicin, cefotaxime, clindamycin, enrofloxacin, cefoxitin, chloramphenicol, tetracycline and trimethoprim/sulphamethoxazole. The 13 multidrug-resistant isolates were isolates that showed clinical resistance to most or all of these antibiotics, whilst the other 13 isolates were sensitive to most or all of the above antibiotics (Table 1).

Bacterial isolates and media used for analysis of the competitive fitness of the multidrug-resistant S. pseudintermedius strain compared with other (non-multidrug-resistant) S. pseudintermedius strains. Among the 13 non-multidrug-resistant isolates, three representative isolates, C001a, C010a and C015, were selected. C007a was used as a representative multidrug-resistant strain. The four S. pseudintermedius isolates used in the competition experiments were isolated from four different dogs within a 3-month period. C001a and C010a were susceptible to at least nine of the antibiotics mentioned above, whilst C015, although sensitive to the other antibiotic classes, was resistant to the β-lactam class of antibiotics (Table 1). Antibiotic-free Luria–Bertani (LB) agar as well as LB agar containing 0.25 μg cefoxitin ml⁻¹ and 1 μg tetracycline ml⁻¹ were used for the selection of the isolates. These antibiotic concentrations were used for selective growth of the isolates, as preliminary experiments indicated that at the above concentrations, susceptible isolates were inhibited whilst the plating efficiency of the resistant strains remained unaffected. A pair-wise growth competition experiment in a nutrient-rich [tryptic soy broth (TSB)] and a defined minimal-nutrient medium (supplemented M9 salts broth) was performed.

Clonal diversity of the S. pseudintermedius isolates by PFGE. The clonal relationship of all S. pseudintermedius isolates was studied by SmalI-PFGE. The PFGE was performed as described previously, using the following running conditions: 6 V cm⁻¹ at 14 °C, with an initial switch time of 2 s and a final switch time of 5 s for 23 h (Murchan et al., 2003; Perreten et al., 2010). The macrorestriction profile was analysed using BioNumerics software version 5.10 (Applied Maths). The similarities were identified using a dendrogram derived from the unweighted pair group method with arithmetic averages and based on Dice coefficients.

Molecular characterization of S. pseudintermedius isolates. PCRs to detect the mecA gene and the virulence genes SIET, lukS, lukF and Panton–Valentine leukocidin (PVL) were carried out as described previously (Futagawa-Saito et al., 2006; Lina et al., 1999; Perreten et al., 2005; Poulsen et al., 2003). For detection of the mecA gene, only the mecA-specific primer set was used. Typing of the staphylococcal cassette chromosome (SCCmec) of the mecA-positive isolates was performed using primers and reaction conditions described previously (Perreten et al., 2010). Typing of the staphylococcal protein A (spa) region of the S. pseudintermedius isolates was performed as described previously (Moodley et al., 2009).

Quantitative biofilm production. An assay for the evaluation of biofilm production by S. pseudintermedius isolates was performed as described previously, with some minor modifications (Futagawa-Saito et al., 2006). Briefly, a single colony from an overnight blood agar culture was cultured overnight in TSB (Oxoid) containing 1 % glucose. Following overnight growth, each culture was diluted 1 : 200 in TSB containing 1 % glucose, before 200 μl of the resulting suspension was inoculated (in triplicate) into each well of a 96-well polystyrene tissue culture plate (BD Bioscience). The inoculated plates were incubated at 37 °C for 18–24 h. The wells were washed twice with 200 μl 0.1 M PBS and dried at room temperature for 5 min. The wells were stained with 200 μl of a 0.1 % safranin solution for 5 min, before washing with 200 μl PBS. The wells were allowed to dry at room temperature for 5 min, before 200 μl 95 % ethanol was added to solubilize the dye present in the adherent biofilm. The absorbance of the adherent biofilm was subsequently measured at 490 nm (A490) in a microplate reader (Beckman Coulter DTX). The result for each strain was reported by subtracting the reading obtained from an experimental ‘blank’. Three independent biological repeats of the experiment were performed.

Resistance to antiseptic agents commonly used in veterinary practices. The MIC of the antiseptic agents chlorhexidine and benzalkonium chloride were determined by broth microdilution as described by the Clinical and Laboratories Standards Institute (previously the National Committee for Clinical Laboratory Standards) (NCCLS, 2000). The minimum bactericidal concentration (MBC) of both antiseptic agents was determined by subculturing the broth dilutions on blood agar following the MIC tests.

Defined minimal medium. The defined minimal medium used for culturing S. pseudintermedius was prepared from a standard commercially available minimal salts M9 broth (Teknova). To support the

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Table 1. Signallment data and antibiogram profile of multidrug-resistant and non-multidrug-resistant *S. pseudintermedius* isolates originating from the surgical wounds of dogs with an SSI

AK, amikacin; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CL, cephalaxin; CN, gentamicin; CTX, cefotaxime; DA, dindamycin; ENR, enrofloxacin; FOX, cefoxitin; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline; R, resistant; S, sensitive; NA, data not available.

<table>
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<tr>
<th>Isolate ID</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Breed</th>
<th>Antimicrobial resistance</th>
<th>mecA</th>
<th>SCC mec</th>
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<td></td>
<td></td>
<td>AK</td>
<td>AMC</td>
<td>AMP</td>
</tr>
<tr>
<td>MDRSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C007a</td>
<td>1</td>
<td>F</td>
<td>Leonberger</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>C017</td>
<td>8</td>
<td>F</td>
<td>English springer spaniel</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>C011</td>
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<td>M</td>
<td>Small breed</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>C025</td>
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<td>F</td>
<td>Cross-breed</td>
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<td>R</td>
<td>R</td>
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<tr>
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<td>R</td>
<td>R</td>
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<tr>
<td>C031b</td>
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<td>M</td>
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<td>S</td>
<td>R</td>
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<tr>
<td>C023b</td>
<td>6</td>
<td>F</td>
<td>Rottweiler</td>
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<td>R</td>
<td>R</td>
</tr>
<tr>
<td>C036</td>
<td>1</td>
<td>M</td>
<td>Jack Russell terrier</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
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<td>C035a</td>
<td>5</td>
<td>F</td>
<td>Irish wolfhound</td>
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<td>R</td>
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<td>C044</td>
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<td>R</td>
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<tr>
<td>C047</td>
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<td>M</td>
<td>Labradoodle</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Non-MDRSP</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C001a</td>
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<td>M</td>
<td>Labrador</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C003a</td>
<td>11</td>
<td>F</td>
<td>German wirehaired pointer</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C002</td>
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<td>M</td>
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<tr>
<td>C004</td>
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<td>F</td>
<td>Cross-breed</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C005</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
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<td>M</td>
<td>Labrador</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C008</td>
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<td>F</td>
<td>Golden retriever</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C010a</td>
<td>7</td>
<td>M</td>
<td>White highland white terrier</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C014</td>
<td>3</td>
<td>M</td>
<td>Newfoundland</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C015</td>
<td>3</td>
<td>M</td>
<td>American bull dog</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<tr>
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<td>M</td>
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<tr>
<td>C019a</td>
<td>4</td>
<td>F</td>
<td>Dogue de Bordeaux</td>
<td>NA</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C003b</td>
<td>11</td>
<td>F</td>
<td>German wirehaired pointer</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>
growth of *S. pseudintermedius* in minimal salts broth, the M9 broth was aeptically supplemented with 2 mM magnesium chloride, 1 mM calcium chloride, 25 μM L-arginine hydrochloride, 50 μM L-glutamic acid, 12.5 μM L-cysteine (adjusted to pH 8.5), 25 μM uridine monophosphate disodium salt, 0.005 % yeast extract and 0.005 % Tween 80. The broth was stored at 4 °C prior to use and used within 2 weeks of preparation. The supplements added to the M9 broth were based on the recommended additives for growth of Gram-positive organisms using a phenotype microarray system (Biolog). TSB was prepared as standard, according to the manufacturer’s guidelines.

**Competition growth experiment.** Prior to the start of the competition experiment, the growth profile of the four competing isolates in TSB and the defined minimal medium was established. For the competition experiment, a single colony of each competing strain was initially incubated for 18 h aerobically at 37 °C in the competition medium. This pre-conditioning step ensured that the competing isolates were acclimatized to the conditions of the study (Lenski et al., 1994). A 0.5 McFarland standard suspension of the two competing strains was prepared and subsequently diluted 1 : 1000 in the appropriate competition broth. The resulting dilution (40 μl) was inoculated into 4 ml of the competition medium, yielding a starting inoculum (of each competing strain) of approximately 10^8 c.f.u. ml⁻¹. The competition experiment was performed at 37 °C for 24 h (with shaking at 200 r.p.m.). Prior to incubation, the inoculated broth was enumerated using a plate counting method, where the competing strains were differentiated based on their susceptibility to cefoxitin or tetracycline. For example, during the growth competition between C001a and C007, the viable count of C001a was determined by subtracting the total number of bacteria on the LB + 0.25 μg cefoxitin ml⁻¹ agar (representing C007a) from the number of bacteria present on the antibiotic-free LB agar (representing both strains). Each competition cycle lasted 24 h, with the competing strains enumerated at the end of each cycle. For subsequent competition cycles, an aliquot of the broth from the previous cycle was diluted 1 : 100 in TSB or minimal broth (as appropriate). A 1 : 100 dilution was used in subsequent competition cycles to ensure the optimum transfer of each competing strain, particularly when one strain was being replaced. A 40 μl aliquot of the resulting dilution was inoculated into 4 ml of a fresh competition broth and incubated as before. Overall, each competition experiment was performed over five cycles, during which the proportions of MDRSP and non-MDRSP were determined at the end of each cycle. Three independent repeats of the competition experiment were carried out, with the difference in fitness between each pair of competing strains (in each experimental repeat) determined by the following equation:

\[
\ln(t^i)_{\text{non-MDRSP}}/\ln(t^i)_{\text{MDRSP}}
\]

\[
\ln(t^0)_{\text{MDRSP/P}}/\ln(t^0)_{\text{non-MDRSP}}
\]

where \( \ln \) is natural log, \( t^i \) is the final bacterial count and \( t^0 \) is the initial bacterial count. As the ratio of MDRSP formed the denominator in the function above, a value below 1 suggested that MDRSP outcompeted the non-MDRSP isolate, whilst a value above 1 indicated that the non-MDRSP isolate outcompeted the MDRSP strain. A value of 1 indicated that the neither of the competing isolates had a fitness advantage in the medium under study. The mean of the three independent experimental repeats represented the relative competitive fitness of the competing strains.

**Statistical analysis.** All data reported here were analysed using a two-tailed Student’s t-test, with \( P < 0.05 \) considered as statistically significant.

## RESULTS

### Clonal relationship of *S. pseudintermedius* isolates cultured from the surgical wounds of dogs

Using an 80 % similarity cut-off value, the 124 isolates produced 74 different patterns, with 67 isolates forming 17 clusters and the remaining 57 isolates (including the 13 antibiotic-sensitive isolates used in the comparative analysis) holding different positions on the dendrogram (Fig. S1, available in the online Supplementary Material). All but one of the 17 clusters contained fewer than six *S. pseudintermedius* isolates, originating from four dogs or fewer. The single cluster with more than six isolates contained 21 *S. pseudintermedius* isolates (including the 13 multidrug-resistant isolates used in the comparative analysis). These 21 isolates originated from 18 different dogs. The PFGE clonal type of the 21 isolates represented the most common *S. pseudintermedius* clonal type observed among the studied cohort.

### Molecular typing and characterization of *S. pseudintermedius* isolates

All 13 MDRSP isolates carried a *mecA* gene, harboured SCC*mec* type II–III and belonged to *spa* type t02. The genotype of the multidrug-resistant *S. pseudintermedius* isolates was thus t02-SCC*mec*II–III. Furthermore, all 13 MDRSP isolates were positive for the leukocidin genes *lukS* and *lukF* and negative for the PVL gene, whilst all but one of the 13 isolates was positive for the SIET gene. All 13 non-MDRSP isolates were positive for the *lukS*, *lukF* and SIET gene and negative for the PVL gene, whilst only one of the 13 non-MDRSP isolates (C015) harboured the *mecA* gene, belonging to SCC*mec* class III. C015 belonged to *spa* type t05.

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### Table 2. Quantitative biofilm production between multidrug-resistant and non-multidrug-resistant isolates of *S. pseudintermedius*

Three independent experimental repeats were performed and the mean of three experiments is shown.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. samples</th>
<th>Mean ( A_{900} )</th>
<th>Range</th>
<th>SEM</th>
<th>SD</th>
<th>95 % confidence interval</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDRSP</td>
<td>13</td>
<td>0.50</td>
<td>0.17–1.02</td>
<td>0.06</td>
<td>0.24</td>
<td>0.36–0.64</td>
<td>0.74</td>
</tr>
<tr>
<td>Non-MDRSP</td>
<td>13</td>
<td>0.47</td>
<td>0.16–0.9</td>
<td>0.06</td>
<td>0.23</td>
<td>0.33–0.61</td>
<td></td>
</tr>
</tbody>
</table>

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Quantitative biofilm production

All 26 S. pseudintermedius isolates were capable of forming biofilms, albeit in different quantities (Table 2). However, there was no statistically significant difference in the level of biofilm production observed between the MDRSP and non-MDRSP isolates ($P=0.74$) (Table 2).

Resistance to commonly used veterinary antiseptics

Commonly used antiseptics in veterinary practices have chlorhexidine and benzalkonium chloride as their active agent. The resistance of the MDRSP and non-MDRSP isolates to these antiseptic agents was determined by determining the MIC and MBC of both sets of isolates to the antiseptic agents. Both MDRSP and non-MDRSP isolates had, on average, a chlorhexidine MIC of $\leq 1 \mu g ml^{-1}$. The corresponding MBC for both sets of isolates was between 1 and 4 $\mu g ml^{-1}$, although one of the non-MDRSP isolates (C004) had an MBC of 8 $\mu g ml^{-1}$. All 13 MDRSP isolates had a benzalkonium chloride MIC of $\leq 1 \mu g ml^{-1}$, with a single isolate (C026) showing an MIC of 4 $\mu g ml^{-1}$. The benzalkonium chloride MBC for all 13 MDRSP isolates was also between 1 and 4 $\mu g ml^{-1}$. Overall, there was no significant difference in the minimum concentration of chlorhexidine and benzalkonium chloride required to inhibit the growth of or kill the MDRSP and non-MDRSP isolates.

Competitive fitness study between MDRSP and three non-MDRSP isolates in a nutrient-rich and a defined minimal medium

The generation times of the four competing isolates (MDRSP and non-MDRSP) when cultured in TSB and minimal-nutrient broth were calculated. In TSB, there was no statistically significant difference ($P=0.8$) in the generation time of the isolates (Table 3). In the minimal-nutrient broth, the isolates exhibited a similar linear growth profile, with no distinct exponential growth phase (Fig. 1). As such, the generation times of the isolates when cultured in the minimal-nutrient broth were not calculated. Based on the above results, it was concluded that the competing isolates were likely to be equally adapted to growth in both competition media.

In the pair-wise competition experiments performed in nutrient-rich medium, the MDRSP isolate (C007a) showed reduced competitive fitness only against C010a, with no appreciable difference in growth fitness observed in the competition experiments with C007a and C001a (Table 4). However, when the competition experiment was carried out in a nutrient-restricted medium, C007a showed reduced growth fitness in the competition experiments with C010a, and also in the competition experiment with C001a (Table 4). Overall, C015 was the only non-MDRSP isolate that did not outcompete C007a in either or both of the growth media used.

DISCUSSION

S. pseudintermedius is an important pathogen that is implicated in SSIs in dogs following orthopaedic interventions. The study carried out here showed an overall heterogeneity in the clonal distribution of S. pseudintermedius commonly implicated in S. pseudintermedius-associated SSIs in dogs. Despite this overall clonal diversity, a single PFGE clonal type, the MDRSP (t02-SCCmecII–III) clonal type, had a relatively high frequency of isolation compared with any other S. pseudintermedius clonal type. In our
study, the MDRSP lineage represented the PFGE cluster with the most number of isolates. With the overall advancement in surgery resulting in patients at greater risk of surgical infections being considered for surgery, there is a greater potential for *S. pseudintermedius*-associated SSIs (NICE, 2008). In light of its prevalence among SSIs (from this study), as well as the spread of the multidrug-resistant ST71-t02-SCCmeCII–III clonal type among dogs in Europe, the MDRSP clonal type is a potential cause for concern in orthopaedic veterinary medicine. The spread and prevalence of this clonal lineage may be because it is more virulent compared with other *S. pseudintermedius* lineages, it is the clonal lineage more likely to contaminate a surgical wound by outcompeting other *S. pseudintermedius* lineages or because it is maintained in different environments through, for example, antibiotic selection. The t02-SCCmeCII–III strain used here was considered to be related to the multidrug-resistant ST71-t02-mecII–III clonal type prevalent among dogs in Europe, as *S. pseudintermedius* with spa type t02 and the SCCmeC II–III genotype is mostly found and associated with isolates of ST71 (Laarhoven et al., 2011).

It is thought that the formation of biofilms and resistance to commonly used veterinary biocides can mediate the survival and persistence of bacteria on different surfaces (Lewis, 2001; Mah & O’Toole, 2001). The theory of persistence on surfaces by the MDRSP clonal lineage may be supported by the fact that meticillin-resistant *Staphylococcus aureus* have, in some studies, shown increased resistance to chlorhexidine compared with meticillin-susceptible *S. aureus* (Kampf et al., 1998; Suller & Russell, 1999). This can be understood in light of the fact that the mechanisms that mediate antibiotic resistance may also mediate resistance to biocides (Poole, 2002; Russell, 1999). However, in our study, we found no evidence to suggest that the multidrug-resistant clonal lineage was any more resistant to chlorhexidine and benzalkonium chloride than other *S. pseudintermedius* clonal lineages. Thus, the MDRSP clonal lineage may be as likely to be eliminated from surfaces cleaned using chlorhexidine- and benzalkonium chloride-based products as other *S. pseudintermedius* strains. Furthermore, the concentration at which chlorhexidine and benzalkonium chloride inhibited or killed both the MDRSP and non-MDRSP isolates in our study (≤ 8 μg ml⁻¹, i.e. ≤ 0.0008 %) is considerably lower than the concentration at which these biocides are generally found in commercial formulations of veterinary disinfectants (e.g. Hibiscrub; 2–4 %, w/v). The role played by the formation of biofilm on surgical implants in *Staphylococcus*-associated SSIs is well documented (Cramton et al., 1999; Donlan, 2001). Whilst biofilm production is thought potentially to contribute to the nosocomial character of meticillin-resistant *S. pseudintermedius* strains (Osland et al., 2012), no significant difference was found in the level of biofilm production between the MDRSP and non-MDRSP isolates (Table 1). Moreover, a recent study that investigated biofilm production among 140 *S. pseudintermedius* isolates (including MDRSP isolates) found that most (96 %) of the isolates analysed produced biofilm, with no significant difference in the level of biofilm production among the isolates studied (Singh et al., 2013).

In exploring specific virulence characteristics of the MDRSP clonal lineage, this study, as well as other recent studies, found no evidence to suggest a difference in the carriage of specific virulence determinants between the MDRSP clonal lineage and other *S. pseudintermedius* clonal types (Gharsa et al., 2013; van Duijkeren et al., 2011). However, it is important to note that these studies investigated specific virulence determinants, and thus it is possible that the MDRSP clonal lineage possesses virulence determinants that were either not investigated or remain hitherto uncharacterized. Nevertheless, a multicentre case–control study investigating the clinical outcome of infections found that MDRSP was no more virulent than other *S. pseudintermedius* strains (Weese et al., 2012).

Clinical outcomes of infections caused by MDRSP, as well as carriage of specific virulence determinants of the MDRSP clonal lineage compared with other *S. pseudintermedius* clonal lineages, have been studied. Despite these studies, a direct growth competition study under different conditions between a member of the MDRSP clonal lineage and other *S. pseudintermedius* lineage has not, to the best of our knowledge, been carried out before. A growth competition study may indicate whether the MDRSP clonal lineage is able to outcompete many other *S. pseudintermedius* clonal lineages, thus explaining its prevalence and spread. In our study, the representative MDRSP isolate studied was outcompeted by at least one of the other three competing isolates in the fitness studies carried out in nutrient-rich and nutrient-restricted media (Table 4). Furthermore, the MDRSP isolate did not outcompete any of the other *S. pseudintermedius* isolates in any of the competition experiments in the nutrient-rich and nutrient-restricted media. This observation suggests that, in the absence of antibiotic-directed selection, there is potential

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**Table 4.** Pair-wise growth competition between a multidrug-resistant and three clonally unrelated non-multidrug-resistant strains of *S. pseudintermedius* in nutrient-rich (TSB) and a defined minimal-nutrient broth

Competitive fitness was calculated using the equation described in Methods.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Competitive fitness of C007a against non-MDRSP isolates (mean ± SEM, n=3)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSB</td>
<td>Minimal-nutrient broth</td>
</tr>
<tr>
<td>C001a</td>
<td>1.060 ± 0.020</td>
<td>1.503 ± 0.072</td>
</tr>
<tr>
<td>C010a</td>
<td>1.533 ± 0.090</td>
<td>3.760 ± 1.63</td>
</tr>
<tr>
<td>C015</td>
<td>1.020 ± 0.045</td>
<td>1.017 ± 0.018</td>
</tr>
</tbody>
</table>
for the MDRSP clonal type to be replaced by non-MDRSP strains in nutrient-rich and particularly in nutrient-restricted environments. The important effect of antibiotic selection pressures in the emergence and spread of the MDRSP clonal lineage is evident from the data obtained from the competition study. This is also substantiated by the lack of a significant difference in the carriage of virulence determinants as well as persistence factors between the MDRSP and other S. pseudintermedius clonal lineages. Unsurprisingly, antibiotic administration is thought to be a risk factor in colonization by meticillin-resistant S. pseudintermedius in dogs (Nienhoff et al., 2011; van Duijkeren et al., 2011). It is vital to note that a general limitation of competition growth studies such as the ones reported here is that the conditions (e.g. nutrition and bacterial diversity) under study may not always accurately reflect the conditions faced by the bacterial strains in vivo or within the environment. This was illustrated by the difference observed in competitive fitness in the nutrient-rich and minimal media. The specific nutrients available to the competing strains, which will vary from host to host, can confer a competitive advantage or disadvantage to an individual strain. As such, it is likely that there are specific environmental and biological conditions that may provide the MDRSP clonal lineage with a competitive advantage in the environment or when in contact with its natural hosts. Interestingly, the only non-MDRSP strain that did not out-compete the MDRSP strain in either of the competition media was the mecA-positive non-MDRSP strain. This observation suggests a fitness cost that may be associated with the presence of the mecA gene. A similar observation has been made in studies on S. aureus, where isolates lacking the mecA gene are able to outcompete their mecA-containing counterparts (Noto et al., 2008). However, this observation was made on only a single strain and therefore needs to be confirmed using a panel of similar strains. Overall, the observations from the studies performed here may be of clinical importance when investigating strategies for controlling the dissemination of the MDRSP clonal type. They indicate a greater urgency on antibiotic stewardship, as well as the potentially important role the use of antiseptics may play in combating the spread of this organism. Furthermore, S. pseudintermedius isolates from dogs are generally clonally diverse (Paul et al., 2012). Therefore, the absence of antibiotic selection pressure within an environment may lead to an overall reduction in the prevalence of the MDRSP clonal type, as an equilibrium that tends towards clonal diversity is likely to be established.

ACKNOWLEDGEMENTS

The company that sponsored the project where the work was carried out is run by NF.

REFERENCES


