Prevalence of methicillin-resistant Staphylococcus aureus among staff and pets in a small animal referral hospital in the UK

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Received 6 June 2005; returned 29 June 2005; revised 20 July 2005; accepted 9 August 2005

Objectives: The occurrence of methicillin-resistant Staphylococcus aureus (MRSA) and the possible relatedness between human and animal isolates were investigated among veterinary staff and hospitalized animals in a referral small animal hospital in the UK.

Methods: A total of 300 swab samples were taken from nasal and oral mucosae of 78 veterinary staff, 45 dogs, 12 cats and from 30 environmental surfaces. Staphylococci were isolated by selective enrichment and characterized by biochemical tests and antimicrobial disc susceptibility testing. MRSA isolates were genotypically confirmed by PCR and typed by PFGE.

Results: MRSA was isolated from 14 staff (17.9%), four dogs (9%), and three environmental sites (10%) yielding a total of 28 MRSA isolates. PFGE analysis revealed that most MRSA isolates were indistinguishable (56%) or closely related (26%) to EMRSA-15, one of the two epidemic MRSA strains dominant in UK hospitals. Like EMRSA-15, the predominant strain isolated from staff, dogs and environmental sites was resistant to fluoroquinolones in addition to all β-lactams.

Conclusions: The study provides evidence of EMRSA-15 mucosal carriage in veterinary staff and hospitalized dogs, with the risk of MRSA carriage in veterinary staff being significantly higher than reported for the UK healthy community. EMRSA-15 was predominant in the hospital environment, including humans, dogs, and inanimate objects, but the mode by which the strain was introduced and spread remains uncertain.

Keywords: antimicrobial resistance, mucosal carriage, dogs, cats, veterinary

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA), a major nosocomial pathogen worldwide, has become endemic in many UK hospitals. The majority of nosocomial isolates are related to two predominant clones, EMRSA-15 and EMRSA-16.1,2 Companion animals, such as dogs, cats and horses, have been implicated more frequently in recent years as potential reservoirs of MRSA.3-5 MRSA infections were initially described sporadically in companion animals but the number of reports has markedly increased in the last few years.6-12 Various case reports have documented MRSA infection in dog owners associated with colonization by genetically related strains in their dogs.4,5,13 Genetically related MRSA isolates have also been reported in horses and in-contact humans.7,14 Transmission of methicillin-susceptible Staphylococcus aureus15 and Staphylococcus intermedius16,17 has been reported between owners and their pets. While S. aureus is the predominant species in humans and horses, S. intermedius represents the vast majority of coagulase-positive staphylococci (CoPS) isolated from skin and mucosae in dogs and cats.18-21 The occurrence of S. aureus in these animals has been reported with frequencies between 1 and 10% of samples.22-27 Similarly, staphylococcal infections in pets are predominantly caused by S. intermedius, with S. aureus representing <10% of clinical staphylococcal isolates from dogs and cats.22,25,27-29 Mucosal carriage of MRSA has been demonstrated in individual pets, horses, and veterinary staff5,10,11,26,30,31 but few data exist on the prevalence of MRSA in these groups.
The aim of this study was to investigate the occurrence of MRSA among veterinary staff, pets and environmental surfaces in a small animal referral hospital in the UK. The epidemiological relationship between human, animal, and environmental MRSA isolates was investigated by PFGE.

Materials and methods

Sampling

Samples were collected on a single day at a university small animal referral hospital (Queen Mother Hospital for Animals, Royal Veterinary College, London, UK). Seventy-eight nurses and veterinary surgeons were sampled during working hours between 9 a.m. and 3 p.m. Forty-five dogs and 12 cats, which were hospitalized, seen as outpatients or kennelled as healthy staff dogs were sampled regardless of their presenting illness or concurrent medication. None of the animals was under treatment for known MRSA infection. The 78 volunteering nurses and veterinary surgeons sampled represented 84.8% of the clinical staff at the hospital, while 100% of dogs and 92.3% of cats were sampled with their owners’ consent. The sampling procedure had been approved by the Royal Veterinary College Ethics Committee. All human samples were provided on a voluntary basis and coded to protect anonymity. The body sites chosen for sampling humans were the median septum of the nose in both nostrils (same swab) and the buccal mucosa at least 1 cm from the lip margins. Animal samples were collected from both nostrils and from the buccal mucosa. Samples were also collected from 30 environmental surfaces (e.g. door handles, desk tops, water bowls) in the waiting area, consulting rooms, procedure rooms and animal wards, where high exposure to hand or animal contact had been observed. Single use cotton swabs on wooden applicators (Technical Service Consultants, Lancashire, UK) were moistened with sterile water and rolled over the sample area (for at least 5 s in staff, dogs and on environmental sites and for at least 2 s in cats).

Isolation and phenotypic characterization of staphylococci

Swab tips were immediately suspended in 10 mL of Tryptone Soya Broth (TSB, Oxoid) containing 10% sodium chloride and incubated for 48 h at 37 °C for selective enrichment of staphylococci. Enrichment cultures were then streaked out on blood agar containing 5% ovine blood (Oxoid) and mannitol salt agar (MSA, Oxoid) for isolation of total staphylococci, and on MSA supplemented with 6 mg/L of oxa-cillin (Sigma) for selective isolation of MRSA. Presumptive staphylococci were identified based on colony morphology, haemolysis, Gram staining and slide coagulation tests. The latter were performed with dog plasma; negative isolates were retested with rabbit plasma. Among CoPS, discrimination between S. aureus and S. intermedius was achieved using the Vogues-Proskauer reaction and a commercial identification system for identification of staphylococci (API ID 32-STAPH, Biomerieux). A sample was recorded as positive for MRSA if one or more colonies of MRSA were identified and one representative colony was chosen from each sample for further testing. The resistance profiles of MRSA, methicillin-susceptible S. aureus (MSSA) and S. intermedius isolates were determined by a disc diffusion method according to the guidelines of the British Society for Antimicrobial Chemotherapy. The following antimicrobial discs were included: co-amoxiclav (3 μg), methicillin (5 μg), clindamycin (2 μg), fusidic acid (5 μg), ciprofloxacin (1 μg), tetracycline (10 μg), co-trimoxazole (25 μg) and mupirocin (5 μg). In addition, cefalexin (Oxoid 30 μg) and ampicillin (Oxoid 2 μg) were included and resistance to these antimicrobials was determined with breakpoints at 12 mm in line with the manufacturers’ recommendations.

PCR for detection of mecA and femB

MRSA isolates were analysed by PCR for the presence of the gene conferring methicillin resistance (mecA) and a gene (femB) used for species identification of S. aureus. One microtitre of extracted DNA (Fast DNA Kit, Qbiogene) was added to a 49 μL volume containing 1× PCR buffer (3 mM MgCl₂), 0.2 mM dNTP, 0.4 μM of specific primers, 1× Q-Solution and 2.5 U HotStarTaq DNA polymerase (Qiagen). The following PCR conditions were used: 15 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 50 °C for mecA (45 °C for femB), and 1 min at 72 °C; and final extension at 72 °C for 10 min.

PFGE

PFGE was performed according to the HARMONY protocol. Briefly, bacterial cultures were grown overnight in Luria-Bertani broth (Difco) and incorporated into agarose blocks (Bio-Rad, Hercules, CA, USA). After bacterial lysis with lysozyme (100 μg/mL) and lysostaphin (50 μg/mL), genomic DNA was digested with Smal (New England BioLabs, USA) and separated on 1% agarose gel using a contour-clamped homogeneous electric field apparatus (CHEF-DRIII) (Bio-Rad, Sweden). The running temperature was set at 14 °C and the voltage was 6 V/cm with an angle of 120° and switching times ranging from 1 to 25 s for a total run time of 20 h. Low-range PFGE Marker (New England BioLabs) was used as the size marker. Reference strains of EMRSA-15 (PM-62) and EMRSA-16 (PM-66) were included in the analysis and their PFGE patterns designated type A and type B, respectively. The epidemiological relationship between isolates from different sources was assessed using the criteria described by Tenover and colleagues.

Statistical analysis

Statistical analysis was done using Windows software Intercooled Stata 7.0 (Stata Corporation, College Station, TX, USA). The data were categorized into two sets of outcomes. Sample outcomes were initially classified into four groups, being samples yielding (1) no isolate, (2) coagulase-negative staphylococci (CoNS), (3) CoPS isolates and (4) CoPS + CoNS isolates (outcome 1). The data were then dichotomized into those yielding MRSA isolates and those yielding non-MRSA or no isolates (outcome 2). A contingency table was used to determine whether there was a significant association between sample source and coagulase results. Multivariate analysis was performed on the variables sample source (human, dog, cat and environment) and sample site (nose and mouth). All data were analysed using Stata’s survey command series, to allow adjustment for clustering within individuals. Multinomial logistic regression analysis was used to model outcome 1 using sample source and sample site as covariates. Binomial logistic regression was used to model outcome 2 using the same covariates.

Results

Prevalence of MRSA and other staphylococci

Twenty-eight methicillin-resistant staphylococci were isolated from 13 staff (nose, mouth or both), four dogs (nose, mouth or both), and three environmental sites (two door handles and a board marker pen). All methicillin-resistant isolates were identified as MRSA based on both phenotypic tests and PCR. Altogether, the prevalence of MRSA was 17.9% among sampled staff, 8.9% among kennelled dogs and 10% among environmental sites. In five staff members, MRSA was isolated from both nose and mouth, in eight only from the nose, and in one only from the mouth. Two dogs hospitalized in the intensive care unit yielded MRSA
from both the nasal and the oral mucosae. Two healthy dogs belonging to MRSA-negative members of the staff yielded MRSA from the oral mucosa only. MRSA was not isolated from any of the cats. None of the sample sources and sites were significantly associated with the occurrence of MRSA.

Staphylococci were isolated from 77 humans (99%), 41 dogs (91%), 10 cats (83%) and 24 environmental sites (80%), yielding a total of 118 CoPS and 162 CoNS isolates. While CoNS predominated among human, feline and environmental isolates, CoPS were more frequently isolated from dogs ($\chi^2 = 48.90$, degrees of freedom $= 9$, $P < 0.001$). Multinomial logistic regression revealed that samples from dogs and cats had a significantly higher risk of yielding CoNS compared with human samples, while samples from cats and the environment had a significantly lower risk of yielding CoPS. Independent of the host, isolation of both CoPS and CoNS was significantly more frequent from nasal samples than from oral samples (relative risk 0.22, 95% CI 0.06–0.74). The isolation frequencies of CoPS, CoNS, MRSA, MSSA and S. intermedius from distinct sample sources and sites are reported in Table 1.

### Antimicrobial resistance patterns

Six patterns of antimicrobial resistance were observed among the 28 MRSA isolates. Nineteen isolates (67.9%), including 10 human isolates and all isolates from dogs and from the environment, showed the same resistance profile as the reference EMRSA-15 (resistance to co-amoxiclav, methicillin and ciprofloxacin). Two isolates, both from the same person, displayed additional resistance to clindamycin. Surprisingly, two isolates from another person appeared to be resistant to methicillin but susceptible to co-amoxiclav after repeated disc diffusion testing. The remaining five isolates were resistant to one or two drugs, in addition to ß-lactams. Resistance to co-amoxiclav was mainly associated with MRSA since MSSA and S. intermedius isolates were rarely resistant to this drug. A similar trend was seen for ciprofloxacin resistance, which was only observed in MRSA (Table 2). All isolates were susceptible to mupirocin.

### PFGE analysis of MRSA isolates

PFGE analysis revealed 10 distinct PFGE types among the 27 MRSA isolates tested (Figure 1 and Table 3). Fifteen isolates (55.6%), including seven human, five canine and three environmental isolates, displayed the PFGE pattern of the reference EMRSA-15 (type A). In addition, four isolates (14.8%), all from staff, were closely related to EMRSA-15 (types C, D and F) and the other three human isolates (11.1%) were possibly related to EMRSA-15 (types E and G) based on the Tenover criteria.37

### Table 1. Isolation frequencies of methicillin-resistant S. aureus (MRSA), methicillin-susceptible S. aureus (MSSA), Staphylococcus intermedius (SI), coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS) from distinct sample sources (human, dog, cat, environment) and sites (nose, mouth)

<table>
<thead>
<tr>
<th>Coagulase type</th>
<th>Group or species</th>
<th>Human</th>
<th>Dog</th>
<th>Cat</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nose</td>
<td>mouth</td>
<td>nose</td>
<td>mouth</td>
</tr>
<tr>
<td>CoPS</td>
<td>MRSA</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>22</td>
<td>8</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>SI + MSSA</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>total CoPS</td>
<td>34</td>
<td>15</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>CoNS</td>
<td>total CoNS</td>
<td>40</td>
<td>52</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>CoPS + CoNS</td>
<td>MRSA + CoNS</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MSSA + CoNS</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SI + CoNS</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SI + MSSA + CoNS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>total CoPS + CoNS</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>None</td>
<td>none</td>
<td>1</td>
<td>11</td>
<td>10</td>
<td>7</td>
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<tr>
<td>Total samples</td>
<td></td>
<td>78</td>
<td>78</td>
<td>45</td>
<td>45</td>
</tr>
</tbody>
</table>

### Table 2. Prevalence (%) of antimicrobial resistance in methicillin-resistant Staphylococcus aureus (MRSA), methicillin-susceptible S. aureus (MSSA) and S. intermedius (SI) isolates

<table>
<thead>
<tr>
<th></th>
<th>AMC</th>
<th>AMP</th>
<th>MET</th>
<th>CLI</th>
<th>LEX</th>
<th>FUS</th>
<th>CIP</th>
<th>TET</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA ($n = 28$)</td>
<td>93</td>
<td>93</td>
<td>100</td>
<td>18</td>
<td>100</td>
<td>4</td>
<td>93</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>MSSA ($n = 46$)</td>
<td>15</td>
<td>85</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>SI ($n = 35$)</td>
<td>0</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

AMC, co-amoxiclav; AMP, ampicillin; MET, methicillin; CLI, clindamycin; LEX, cefalexin; FUS, fusidic acid; CIP, ciprofloxacin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole.
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Figure 1. PFGE types observed among methicillin-resistant *Staphylococcus aureus* isolates. Lanes: 1, low-range PFGE marker; 2, PFGE type A, reference isolate for EMRSA-15; 3, type B, reference isolate for EMRSA-16; 4, type C; 5, type D; 6, type E; 7, type F; 8, type G; 9, type H; 10, type I; 11, type J; 12, type K.

Table 3. Genetic relatedness and distribution of PFGE types in methicillin-resistant *Staphylococcus aureus* of human, canine and environmental origin

<table>
<thead>
<tr>
<th>PFGE type</th>
<th>Relatedness to type A</th>
<th>Human (n = 18)</th>
<th>Canine (n = 6)</th>
<th>Environmental (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>unrelated</td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>closely related</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>closely related</td>
<td>2(^{b})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>closely related</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>possibly related</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>closely related</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>possibly related</td>
<td>2(^{b})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>unrelated</td>
<td>1</td>
<td>1(^{b})</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>unrelated</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>unrelated</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K</td>
<td>unrelated</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a}\)According to the criteria for interpretation of PFGE proposed by Tenover and colleagues.\(^{35}\) Closely related, the differences between the PFGE profiles could result from a single genetic event (one point mutation or one insertion); possibly related, the differences could result from two genetic events (two insertions).

Overall, 81.5% of the MRSA isolates were genetically related to EMRSA-15, including 14 of the 18 human isolates and five of the six canine isolates. One canine isolate and four human isolates showed PFGE patterns unrelated to type A (H, I, J and K). Type B (EMRSA-16) was not detected.

Discussion

The epidemic clone EMRSA-15 was shown to occur frequently in staff, patients and environmental sites in the referral small animal hospital. To the best of our knowledge, this is the first study demonstrating the occurrence of EMRSA-15 in dogs and veterinary staff. This finding is particularly noteworthy in light of the clinical importance of EMRSA-15 as a causative agent of nosocomial bacteraemia in the UK. It has been estimated that EMRSA-15 causes ~60% of cases of MRSA bacteraemia in UK hospitals. In 2003, 19 244 cases of bacteraemia caused by *S. aureus* were reported in England alone and MRSA accounted for 39% of the total reports.\(^{39}\) The recovery of EMRSA-15 from dogs and veterinary staff is of epidemiological significance since it indicates that this important nosocomial pathogen is not confined to hospitals and can be propagated by healthy humans and animals in the community.

Comparison of MRSA carriage rates reported by different studies is problematic since various sampling strategies and isolation methods can be used for assessing staphylococcal carriage. However, the rate of MRSA mucosal carriage observed in this study among veterinary staff (18%) far exceeds those reported in UK community surveys (1.5% and 0.78%).\(^{39,40}\) MRSA nasal carriage was previously reported in three of five veterinary staff during an MRSA outbreak in an equine hospital in the USA,\(^{7}\) and in 13% of 107 personnel in contact with MRSA-positive horses in Canada.\(^{31}\) Thus, similarly to human healthcare workers (6.2% in France, 6% in Turkey)\(^{31,42}\) veterinary staff may represent a category at risk for MRSA carriage.

The observed mucosal MRSA carriage in dogs (9%) also appears to be high but comparable data for dogs are not available. It should be considered that the use of selective enrichment procedures, like those used in this study, allows MRSA detection even if bacterial numbers are low, therefore leading to higher carriage rates in comparison with studies based on random isolation. Although risk factors were not investigated in this study, two of the four MRSA-positive dogs were hospitalized in the intensive care unit, suggesting that risk factors associated with human MRSA carriage may apply to dogs. Antimicrobial therapy, especially with cephalosporins and fluoroquinolones,\(^{43,44}\) is a well-recognized risk factor for MRSA selection in humans.\(^{45}\) and may also provide an avenue for acquisition of MRSA by dogs after their natural flora has been wiped out. This possibility is supported by the lack of concurrent isolation of *S. intermedius* in all four MRSA-positive dogs. Both cephalosporins and fluoroquinolones are frequently used in dogs, especially for treatment of skin infections caused by *S. intermedius*.\(^{46}\)

The lack of MRSA detection in the cats under study is unlikely to be due to host specificity of *S. aureus* since MSSA was found at similar frequencies in dogs and cats. This result could be influenced by factors other than host specificity, such as differences in antimicrobial use, i.e. fluoroquinolones and cephalosporins are more frequently used in dogs, or environmental factors, i.e. cats were hospitalized in a ward separate from the dogs, although treatment and diagnostic facilities were shared and staff attended to both species. Alternatively, physical contact of humans with cats may be less intense than with dogs, possibly making transfer of resistant organisms less frequent. The prevalence of MRSA at environmental sites was very similar to that previously reported for a Canadian equine hospital.\(^{47}\) These data emphasize the need for MRSA surveillance in veterinary hospitals.
The origin of EMRSA-15 isolates in the small animal hospital remains unknown. Various authors have proposed that MRSA isolated from companion animals originate from humans. However, the relatively frequent isolation of MRSA from veterinary staff suggests that daily exposure to companion animals may represent a risk factor for MRSA carriage. Alternatively, other aspects related to the veterinary profession, e.g. handling of antibiotics and disinfectants, could explain this observation. From an evolutionary point of view, it has been proposed that S. aureus could have acquired mecA from Staphylococcus sciuri, a species frequently occurring in animals and harbouring a close structural homologue of mecA in the chromosome. Animal isolates genetically distinct from epidemic MRSA clones, like the one found in this study (PFGE type H), have been reported in previous studies.

Atypical genetic lineages of MRSA may arise in animals by horizontal transfer of mecA from CoNS to MSSA. The gene has also been identified in S. intermedius isolates from dogs and in CoNS and coagulase-variable staphylococci from dogs, cats and horses. This raises the possibility of animals being a reservoir of unusual MRSA isolates that could then be spread to humans.

In addition to the zoonotic potential of staphylococcal infections, there is concern about the emergence of antimicrobial resistance amongst isolates of staphylococci from companion animals. Increasing resistance has been observed to various antimicrobials frequently used in veterinary practice including some broad-spectrum drugs and preparations used in human medicine.

Methicillin resistance is mediated by a penicillin-binding protein with low affinity to β-lactam antibiotics (PBPs 2A) encoded by mecA. Phenotypic detection of methicillin resistance is difficult due to heterogeneous expression of mecA gene by many staphylococcal strains. Expression of resistance is affected by the testing conditions, including the β-lactam drug tested for detection of resistance. Therefore, the unusual susceptibility of the two human MRSA isolates to co-amoxiclav may be due to lack of expression of mecA in the presence of amoxicillin. Susceptibility to co-amoxiclav has previously been reported in an MRSA isolate from a dog.

In conclusion, the high prevalence of MRSA carriage among veterinary staff and dogs was unexpected. Risk analysis studies are needed to determine whether veterinarians, and people living or working with companion animals, are categories at risk for MRSA carriage. Monitoring of MRSA in animals should be promoted in veterinary surveillance programmes on antimicrobial resistance to elucidate the possible contribution of companion animals to the spread of MRSA in the community. If necessary, adequate measures, e.g. treatment of animal carriers with topical antibiotics, could be taken to eradicate MRSA in animals and to prevent this important human pathogen from becoming endemic in the animal population.

Acknowledgements

We are grateful to Maggie Bushnell and Ruth Gravelle, who processed the broth samples and performed the antimicrobial susceptibility tests. Christine Young prepared the agar plates. Sue Shaw performed the sampling of veterinary staff. We are grateful to Tony Bønneleycke who performed PFGE typing at The Royal Veterinary and Agricultural University. This study was partly supported by Royal Veterinary College, University of London, internal research funds.

References


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