Isolation rates of meticillin-resistant *Staphylococcus aureus* in dogs, cats and horses in Ireland


A retrospective analysis and prospective surveillance study were conducted to determine isolation rates of meticillin-resistant *Staphylococcus aureus* (MRSA) in dogs, cats and horses in Ireland. Clinical samples that had been submitted to University College Dublin (UCD) for routine microbiological examination over a four-year period (2003 to 2006) were analysed in the retrospective analysis, which included clinical samples from 3866 animals. In the prospective surveillance study, samples from healthy animals presenting for elective surgery as well as from animals with a clinical presentation suggestive of MRSA infection were investigated. Animals attending 30 veterinary practices throughout Ireland and a similar population of animals presented to UCD were studied. The isolation rates for animals in the retrospective study were 1.1 per cent (32 of 2864) for dogs, 0.7 per cent (four of 619) for cats and 5.2 per cent (20 of 383) for horses. The overall isolation rate of MRSA was 1.4 per cent (56 of 3866).

Isolation rates for healthy animals in the prospective study were 0.4 per cent (one of 286) for dogs and 1.7 per cent (four of 236) for horses; MRSA was not isolated from cats (0 of 47). Isolation rates for animals suspected of being infected with MRSA were 8.1 per cent (14 of 173) for dogs and 4.6 per cent (three of 65) for horses; MRSA was not isolated from cats (0 of 47).

Since its first isolation in 1961, meticillin-resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial pathogen worldwide (Voss and Doebbeling 1995). Primarily associated with hospital-acquired infections, MRSA is now seen with increasing frequency in the community (Vandenesch and others 2003). Reports of animals with MRSA-associated infections have been documented in many countries in recent years (Seguin and others 1999, Duquette and Nuttall 2004, Cuny and others 2006). The first confirmed clinical case in Ireland was described in 2003 (O’Mahony and others 2005). A number of reports have suggested that the acquisition and transmission of MRSA infections in pets are linked to human sources (Seguin and others 1999, Rich and Roberts 2004, O’Mahony and others 2005, Leonard and others 2006).

A study from the USA reported that four of 36 (11 per cent) *S aureus* infections in dogs and four of 18 (22 per cent) in horses were MRSA infections (Middleton and others 2005). Those authors also noted that one of two cats with *S aureus* infections was MRSA-positive. A 12-month study in the UK reported 95 MRSA-positive cases, which consisted of 69 dogs, 24 cats, one rabbit and one horse (Rich and Roberts 2004). Reports investigating the prevalence of colonisation of healthy animals in the community included a study of healthy dogs in Slovenia, in which no MRSA carriage was detected (Vengust and others 2006), a study in the UK, in which one carrier was found among 255 healthy dogs (0.4 per cent) (Rich and Roberts 2006) and a study in Canada, in which one carrier was found among 193 healthy dogs (0.5 per cent) (Hanselman and others 2006).

The present study investigated isolation rates of MRSA retrospectively among samples submitted to a veterinary diagnostic microbiology laboratory in a tertiary care centre/hospital, and prospectively among healthy pets and horses in the community in Ireland, and among clinically affected animals attending first-opinion veterinary clinics and a tertiary care centre/hospital.

Materials and methods

The Diagnostic Veterinary Bacteriology Laboratory (DVBL) at University College Dublin (UCD) provides a diagnostic service not only for cases attending the University Veterinary Teaching Hospital (UVTH) but also for cases attending private veterinary practices throughout Ireland. The laboratory processes clinical samples from both companion and food-producing animals.

Isolation rates of MRSA in dogs, cats and horses were investigated during three study periods. The investigation was approved by the UCD Animal Research Ethics Committee, and the consent of the animal owners was obtained before obtaining screening samples.

Study 1

MRSA isolation rates were investigated retrospectively by analysing the numbers of MRSA-positive samples among all clinical samples submitted for routine microbiological investigation from pets (dogs and cats) and horses over the four-year period from 2003 to 2006. Samples were received from cases attending the UVTH and from external cases (that is, animals attending private veterinary practices throughout Ireland). Only the first isolate obtained for each animal...
was recorded. The total number of MRSA-positive animals detected during the period and the numbers detected in each year were calculated. Statistical comparison of this population of animals was not carried out because the populations involved were so diverse. Criteria for the submission of samples for culture from primary veterinary practices and tertiary referral hospitals might also have varied.

Study 2
A prospective study was carried out to investigate MRSA isolation rates in both healthy pets and horses, and those presenting with clinical signs suggestive of MRSA infection. Animals were presented to 30 veterinary practices located throughout Ireland during an eight-month period between October 2005 and May 2006. The participating practices were located in each of the four Irish provinces, with 14 practices in Leinster, 11 in Munster, three in Connacht and two in Ulster. Practices were requested to submit samples from up to 20 cases (one sample for each animal) from consecutive animals in either of two populations of animals that attended their clinics. The first population of animals comprised clinically healthy dogs and cats attending a practice for routine elective surgery; the veterinary practitioner considered these animals to be infection-free (for the purposes of the present study these animals are referred to as ‘healthy’). Healthy animals were screened by culture of nasal samples obtained using specialised narrow Amies nasal transport swabs (Copan). Each swab was inserted approximately 1 to 2 cm into the nares and rotated in both nasal passages. Nasal samples were also collected from 129 clinically healthy horses from various sources: from five stud farms (68 horses), 51 individually owned horses, and horses attending hunt meetings (three horses) and race meetings (seven horses).

The second population of animals comprised animals with a clinical history or presentation that the veterinary practitioner considered to be suggestive of MRSA infection (referred to as ‘infected’ cases). Criteria for case selection in this group included animals with a discharging wound following an orthopaedic procedure, other types of discharging wounds, purulent skin conditions and clinical conditions suggestive of respiratory tract infection. Clinical samples only were submitted from this group of animals. Samples from infected cases were obtained using Amies charcoal transport swabs (Copan).

Study 3
A prospective study was undertaken to investigate MRSA isolation rates in two groups of animals attending the UVTH. These were healthy and infected animals that presented consecutively as cases. As with study 2, nasal swabs were submitted from healthy animals and only clinical samples were submitted from the infected group. Only isolates from the first sample from an animal were included. Dogs and cats were screened between October 2005 and May 2006; horses were screened between February and October 2006.

Salt enrichment culture study
A total of 662 consecutive clinical samples received between April 2005 and December 2006 were cultured by direct culture on to blood agar (Columbia blood agar base; Oxoid) supplemented with 5 per cent defibrinated sheep blood, and by salt enrichment in salt tryptone soya broth (Oxoid) supplemented with 7.5 per cent (w/v) sodium chloride, and the numbers of MRSA-positive samples detected by each method were compared.

Sample processing
Samples were cultured directly on to blood agar. From April 2005, in addition to direct culture, all samples were cultured using salt enrichment. Following incubation for 18 hours at 37°C, broths were subcultured on to blood agar containing Staphylococcus/ Streptococcus selective supplement (SR79E; Oxoid), containing colistin and nalidixic acid, and incubated overnight at 37°C. Colonies were identified as Staphylococcus aureus by morphology, Gram stain, the catalase test, the slide coagulase test, the latex agglutination test (Pastorex Staph-Plus, Bio-Rad) and ability to ferment maltose in Purple Agar Base (BD Difco) supplemented with 1 per cent (w/v) maltose (Raus and Love 1985). All coagulase-positive staphylococci were identified to species level using one of two kits (API Staph or API Rapid ID 32 Staph, bioMérieux). Meticillin resistance was detected by determining resistance to 30 µg cefoxitin (Oxoid) using the Clinical and Laboratory Standards Institute disc diffusion method (CLSI 2005), and confirmed by detecting penicillin-binding protein 2a using the Mastalex-MRSA kit (Mast Diagnostics).

Results
Salt enrichment culture evaluation
Of the 662 clinical samples processed by direct and salt enrichment culture during the pilot study, 35 samples were found to be MRSA-positive (5.3 per cent). Direct culture yielded MRSA from 27 samples (4.1 per cent), eight samples found to be negative by direct culture were positive by salt enrichment culture, and one sample was positive by direct culture but negative by salt enrichment culture. The inclusion of salt enrichment culture enhanced the rate of detection of MRSA by 23.0 per cent.

Study 1
Between 2003 and 2006, the DVBL processed 5653 clinical samples from 3266 animals, comprising consecutive samples from 2864 dogs, 619 cats and 383 horses. MRSA was isolated from 56 animals (1.4 per cent). Isolation rates of MRSA from animals attending the UVTH and from those attending private veterinary practices (external cases) are shown in Table 1. MRSA was detected in 32 of 2264 dogs (1.1 per cent), four of 619 cats (0.65 per cent) and 20 of 383 horses (5.2 per cent). Most MRSA-positive samples were derived from wound sites (26 dogs, two cats and 20 horses). The remaining MRSA-positive samples were urine samples from two cats and one skin sample, one joint sample, one blood sample, two nasal samples and one tracheostomy tube sample from six dogs.

The isolation rate of MRSA in dogs was higher for external cases (1.5 per cent [23 of 1509]) than for UVTH submissions (0.7 per cent [nine of 1355]). MRSA isolation rates in cats from external and UVTH samples were 0.5 per cent (two of 407) and 0.9 per cent (two of 212), respectively. Isolation rates in horses from external and UVTH samples were 7.8 per cent (five of 64) and 4.7 per cent (15 of 319), respectively.

A marked increase in MRSA detection rates was observed among external equine cases in 2005 as a consequence of a major outbreak on one stud farm. Three equine cases in 2003 were attributable to a cluster of cases that occurred at the UVTH, from which the index case was a referral from the same stud farm. Another cluster of five cases occurred at the UVTH in 2004, where the index case was also referred from this stud farm. The four MRSA-positive horses at the UVTH in 2005 were all cases referred from this stud farm; the samples from the five external equine cases in 2005 were submitted by an external practitioner working for the stud farm. In total, 11 horses from the stud farm were identified as MRSA-positive over the four-year period, and the remaining two and four cases in 2003 and 2004, respectively, were linked to this prolonged outbreak.

Study 2
A total of 503 animals were sampled during study 2. Among nasal samples from 290 healthy animals, the overall MRSA isolation rate was 1 per cent (three of 290), from 0.8 per cent of dogs (one of 133)

### TABLE 1: Isolation rates of meticillin-resistant *Staphylococcus aureus* (MRSA) in samples from animals presented to the University Veterinary Teaching Hospital (UVTH), Dublin, or private veterinary practices throughout Ireland during 2003 to 2006 (study 1)

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Cats</th>
<th>Horses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UVTH Private</td>
<td>UVTH</td>
<td>UVTH Private</td>
</tr>
<tr>
<td>2003</td>
<td>7/460 (1.5)</td>
<td>1/259 (0.4)</td>
<td>0/125</td>
</tr>
<tr>
<td>2004</td>
<td>5/353 (1.4)</td>
<td>1/336 (0.3)</td>
<td>1/103 (1.0)</td>
</tr>
<tr>
<td>2005</td>
<td>5/324 (1.5)</td>
<td>2/317 (0.6)</td>
<td>1/89 (1.1)</td>
</tr>
<tr>
<td>2006</td>
<td>6/372 (1.6)</td>
<td>5/448 (1.1)</td>
<td>0/96</td>
</tr>
<tr>
<td>Total</td>
<td>23/1509 (1.5)</td>
<td>9/1355 (0.7)</td>
<td>2/407 (0.5)</td>
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</table>
and 1.6 per cent of horses (two of 129) (Table 2). A total of 213 wound swabs from the infected group yielded MRSA-positive results from 4.7 per cent of samples (10 of 213). All of these MRSA-positive samples came from the 143 dogs studied (7.0 per cent [10 of 143]) (Table 2). The two healthy horses that were positive for MRSA carriage came from different parts of the country (one from Connaught and one from Leinster). All 67 samples from cats were MRSA-negative (Table 2).

Study 3

This prospective study investigated samples from 351 animals attending the UVTH. MRSA was recovered from two of the 279 nasal swabs from healthy animals (0.7 per cent). Both MRSA-positive samples were from horses (Table 2). In the infected group, 9.7 per cent of samples (seven of 72) were MRSA-positive. MRSA was recovered from 13.3 per cent of dogs (four of 30) and 0.8 per cent of horses (three of 34) (Table 2). All samples from cats were MRSA-negative.

Overview

Altogether, across all three studies, samples from 4682 animals were investigated. These comprised 3866 samples in study 1, 503 in study 2 and 351 in study 3; however, 38 infected cases in study 3 (shown in Table 2) were also included in study 1. MRSA was detected in 74 per cent of these 4682 animals (1.5 per cent). 0.9 per cent of healthy animals (five of 569) carried MRSA and 1.7 per cent of animals (69 of 4113) in the infected group were MRSA-positive. Salt enrichment enhanced detection of MRSA in 11.6 per cent of animals with clinical infection (eight of 69) and in 50 per cent of carri
ers (four of five). The 74 MRSA-positive animals resided in 19 of the 32 counties in Ireland.

Discussion

The aim of the present study was to investigate the isolation rates of MRSA from pets (cats and dogs) and horses attending primary care veterinary clinics and a tertiary referral veterinary hospital in Ireland. In keeping with common practice in many parts of the world (Morris and others 2006), veterinary practitioners in Ireland do not usually send samples for routine culture and susceptibility testing unless there is evidence to suggest that animals are not responding to antimicrobial therapy. Furthermore, clinical cases referred to UVTH as a tertiary referral centre may often be complex or have failed to respond to antibiotic therapy. In the present study, it is likely that studies 1 and 3 (which investigated cases attending UVTH and samples sent to UVTH from external practitioners) may have had an element of sampling bias in favour of animals that had already received antimicrobial therapy. Such a bias would not have been present in study 2, in which samples were sent from consecutive cases in general practice. Previous antimicrobial use is a recognised risk factor for selection of antibiotic-resistant bacteria, and although the extent of this bias in studies 1 and 3 cannot be quantified, the cases investigated in these studies may represent the animals at greatest risk for MRSA. A sampling bias may also exist in study 1, as the criteria for sample submission for culture may vary between primary practices and tertiary veterinary hospitals. There may have been a potential age bias in study 2, as healthy animals might have tended to be younger due to animals attending for elective procedures such as neutering and vaccination.

The investigation of healthy horses showed that four animals were MRSA-positive: two were horses attending the UVTH, one was a referral from the University Veterinary Teaching Hospital (UVTH), Dublin (study 3) and one was a referral from a veterinary practice in Leinster. All 67 samples from cats were MRSA-negative.

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There is great variation in the reported rates of MRSA among the different animal species. Rates among healthy dogs generally range from 0.4 per cent (one of 255) to 0.7 per cent (six of 815) in the UK and Hong Kong, respectively (Rich and Roberts 2006, Boost and others 2007). An unusually high rate of 9 per cent (four of 45) among healthy dogs was recorded at an institution in the UK, in which 16 per cent (13 of 78) of staff and 10 per cent of environmental samples were MRSA-positive (Loeffler and others 2005). A German study investigating diagnostic samples from animals attending a veterinary teaching hospital reported that 56.2 per cent (18 of 32) of Staphylococcus aureus isolates from dogs were meticillin-resistant (Winther and others 2008). In that study, the authors noted a possible case load bias and suggested that the figures might not represent the true proportion of MRSA found in routine submissions to a veterinary diagnostic laboratory.

In the present study, surveillance of healthy pets and horses revealed rates of MRSA carriage in dogs of 0.8 per cent (one of 133) and in horses of 1.6 per cent (two of 129) (Table 2). No MRSA carriage was detected in healthy dogs or cats attending the UVTH or in cats attending external practices. The rate detected among healthy dogs in the present study is comparable to the 0.7 per cent (six of 815) rate reported in Hong Kong, where healthy dogs attending veterinary practices were screened for MRSA carriage (Boost and others 2007). In study 1, there was a higher rate among dogs from external cases (1.5 per cent [23 of 1509]) than from cases attending the UVTH (0.7 per cent [nine of 1355]). The higher rate among external canine cases may be attributable to four external veterinary practices having clusters of MRSA cases among animals presented for veterinary attention during the four-year study period. Of the 23 MRSA-positive external cases, 16 were associated with four practices, which included one practice that experienced two outbreaks involving nine dogs over the four-year period (Leonard and others 2006). Of the nine MRSA cases referred to the UVTH, seven were referred by different veterinary practices located in different counties around Ireland, and single MRSA-positive cases only were associated with these practices over the four-year period. One of the other two referrals was from a practice that had identified a MRSA-positive clinical canine case and three staff members that were identified with MRSA carriage two months before the commencement of the present study. The other practice had a second MRSA-positive case confirmed five months after completion of the present study. The MRSA cases in cats (four of 619) in the present study were single isolated cases, and none of the cases was associated with an MRSA outbreak. In the literature, MRSA-associated infection in cats appeared to be relatively rare compared with canine cases (Boag and others 2004, Abraham and others 2007).

Among canine cases with suspected clinical infection (studies 2 and 3), the MRSA isolation rates were 7 per cent (10 of 143) from the 30 participating practices and 13 per cent (four of 30) in the UVTH. These high rates indicate a potentially serious problem with MRSA in veterinary medical practice in Ireland. The 10 external MRSA-positive infected cases attended five of the 30 practices that took part in the surveillance study. These five practices were located in five different counties. Three of these practices had previous histories of MRSA infections involving pets, and it is possible that transmission to and acquisition of MRSA in these new cases is attributable to MRSA colonisation of veterinary staff or to environmental contamination in the practices. The four MRSA-positive canine cases in the UVTH (study 3) were referred to the UVTH from four different clinics in four different counties. These clinics had not participated in study 2 and had no known history of or association with MRSA.

The investigation of healthy horses showed that four animals were MRSA-positive: two were horses attending the UVTH, one was a referral from the University Veterinary Teaching Hospital (UVTH), Dublin (study 3) and one was a referral from a veterinary practice in Leinster. All 67 samples from cats were MRSA-negative.

<table>
<thead>
<tr>
<th>Study</th>
<th>Dogs</th>
<th>Horses</th>
</tr>
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<tbody>
<tr>
<td>Healthy</td>
<td>Infected</td>
<td>Healthy</td>
</tr>
<tr>
<td>Study 2 (n=503)</td>
<td>1/133 (0.75)</td>
<td>10/143 (7.0)</td>
</tr>
<tr>
<td>Study 3 (n=351)</td>
<td>0/153</td>
<td>4/30 (13.3)</td>
</tr>
<tr>
<td>UVTH</td>
<td>1/286 (0.35)</td>
<td>14/173 (8.1)</td>
</tr>
<tr>
<td>Total</td>
<td>2/107 (1.9)</td>
<td>3/34 (8.8)</td>
</tr>
</tbody>
</table>
The isolation rate of 7.5 per cent (15 of 201) from wound samples from dogs, and open wounds (Seguin and others 1999, Tomlin and others 1999, MRSA outbreak described in study 1. was referred from the equine stud farm that was involved with the infection. By coincidence, one of the cases that had not participated in study 2. By coincidence, one of the cases was referred from the equine stud farm that was involved with the outbreak described in study 1. 

The rates of MRSA infection in horses in study 1 were 7.8 per cent (five of 64) among external equine cases and 4.7 per cent (15 of 319) in UVTH cases, which related to the previously described outbreak on a stud farm (O’Mahony and others 2005). The prospective studies (studies 2 and 3) showed no MRSA among 31 animals attending the veterinary hospital. In that study, 4.5 per cent (three of 67) of animals had MRSA-associated infections (Baptiste and others 2005). Twelve staff at the hospital were screened for MRSA carriage using direct culture and none was MRSA-positive. Weese and others (2005a) documented MRSA carriage rates in horses following outbreaks at a Canadian veterinary hospital and farm. In 2000, they found that 10 per cent of staff (two of 21) and 4 per cent of horses (two of 57) at the veterinary hospital were carriers of MRSA and, in 2002, 12 per cent of staff (15 of 127) and 8 per cent of horses (25 of 320) were MRSA-positive. Those authors also reported that, in 2002, the MRSA carriage rates among staff and horses at an equine stud farm were 12 per cent (eight of 68) and 13 per cent (41/321), respectively. The three MRSA-positive UVTH equine cases in the present study (in study 3) were referred to the UVTH by three different clinics that had not participated in study 2. By coincidence, one of the cases was referred from the equine stud farm that was involved with the MRSA outbreak described in study 1. 

The isolation rate observed in infected cases of dogs and horses attending the UVTH may be attributable to a heightened awareness among veterinary practitioners of the need to refer such cases to a tertiary referral clinic for investigation. The majority of MRSA infections in pets and horses are associated with postoperative infections and open wounds (Seguin and others 1999, Tomlin and others 1999, Rich and Roberts 2004). Walther and others (2008) reported an MRSA isolation rate of 7.5 per cent (15 of 201) from wound samples from dogs, a figure comparable to the 7 per cent rate observed in study 2. Cuny and others (2006) reported that in 24 horses with MRSA-associated infections, 14 positive samples were from wound-affected sites. There are numerous reports in the literature describing approaches to optimise the sensitivity of culture methods for MRSA, which have been reviewed comprehensively by Brown and others (2005) and incorporated into guidelines for the laboratory diagnosis of MRSA. These guidelines recommend the inclusion of an enrichment step. Even with the availability of selective chromogenic media, which are more sensitive than previous conventional selective media, enrichment culture further increased sensitivity (Nonhoff and others 2009). A recent study evaluating a rapid real-time PCR assay for the detection of MRSA compared the assay results with results obtained from direct culture on chromogenic agar and enrichment culture, and reported that the use of enrichment culture increased the number of culture-positive samples by 51 per cent (Rossney and others 2008). The present study, in which the use of salt tryptone soya broth enhanced the yield of MRSA by 23 per cent (eight of 35), provides further evidence of the importance of enrichment culture.

Since 2003, the DVBL has identified MRSA-associated infections in pets and in horses from locations throughout Ireland. In addition, prospective studies have documented MRSA colonisation in healthy pets and horses in the community in Ireland. MRSA colonisation in healthy companion animals is a cause for concern for both human and veterinary medicine, and highlights the need for good-quality infection control in veterinary practices and veterinary hospitals. The present study provides essential groundwork for future research into MRSA carriage and infection in animals, as well as providing information of importance to veterinary practitioners in Ireland.

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