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Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland

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Abstract

Reports of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals have become more frequent in recent years. This paper documents the recovery of MRSA from animals with respiratory, urinary tract or wound infection and from animals subjected to surgical procedures following treatment in one veterinary hospital and 16 private veterinary clinics in different geographical locations throughout Ireland. MRSA was recovered from 25 animals comprising 14 dogs, eight horses, one cat, one rabbit and a seal, and also from 10 attendant veterinary personnel. Clinical susceptibility testing suggested that the 35 isolates fell into two different groups. One group of isolates (Group 1) was resistant to one or more of the following classes of antimicrobials: macrolides, lincosamines, tetracyclines and/or fluoroquinolones. The second group (Group 2) was resistant to macrolides, aminoglycosides, tetracyclines and trimethoprim/sulphamethoxazole and variably resistant to fluoroquinolones, lincosamines and rifampicin. One isolate in Group 2 was susceptible to trimethoprim. Epidemiological typing by antibiogram-resistogram (AR) typing, biotyping and by chromosomal DNA restriction fragment length polymorphism analysis using *Sma*I digestion followed by pulsed field gel electrophoresis (PFGE), confirmed these two major clusters. PFGE analysis showed that most isolates from non-equine animals were indistinguishable from each other and from the isolates from personnel caring for these animals. MRSA was isolated from eight horses which attended six different veterinary practices before referral to an equine veterinary hospital. Isolates from the eight horses and from their attendant personnel had PFGE patterns that were indistinguishable and were unlike the patterns obtained from the other isolates. Comparison of PFGE patterns of isolates from veterinary sources with patterns from MRSA recovered in human hospitals showed that the most frequently occurring pattern of MRSA from non-equine animals was indistinguishable from the predominant pattern obtained from the most prevalent MRSA strain in the human population in Ireland. However, the patterns of the isolates from horses were unlike any patterns previously reported in Irish studies of human isolates. This study shows that transmission of two strains of MRSA is occurring in veterinary practices in Ireland and that one strain may have arisen from human hospitals. The source of the second strain remains to be determined.
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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in the United Kingdom (UK) in 1961 and by the mid-1990s had become a major problem worldwide (Anon., 1998). In Ireland during 2002, 416 of 998 (42%) *S. aureus* isolates from human patients with bacteraemia were resistant to methicillin, placing Ireland fourth highest of 27 European countries submitting data to the European Antimicrobial Resistance Surveillance System (EARSS) (Anon., 2003).

Isolation of MRSA from animals was first reported in 1972 following its detection in milk from mastitic cows (Devriese et al., 1972). Occasional reports have been published of MRSA infection in domestic animals including dogs, cats, cattle, sheep, chickens, rabbits and horses, but in recent years, the number of cases appears to be increasing (Devriese and Hommez, 1975; Hartmann et al., 1997; Pak et al., 1999; Tomlin et al., 1999; Lee, 2003; Goni et al., 2004; Rich and Roberts, 2004; Weese et al., 2004a). Two outbreaks of infections in separate veterinary teaching hospitals in the United States of America (USA) and Canada have been recently documented (Seguin et al., 1999; Weese et al., 2004a). The American study suggested that the veterinary hospital staff were the primary source of infection although the mode of transmission was unclear, whereas the Canadian study concluded that environmental contamination played a significant role, in particular contamination of stalls housing MRSA-positive horses.

Epidemiological typing forms an important part of the investigation of outbreaks of MRSA infection. Chromosomal DNA analysis using the restriction endonuclease *Sma*I followed by pulsed field gel electrophoresis (PFGE) is the current 'gold standard' for DNA fingerprinting of MRSA and is widely used (Murchan et al., 2003). Other studies have shown that antibiogram-resistogram (AR) typing using a panel of 23 antimicrobial agents is a useful phenotypic method (Rossney et al., 1994b, 2003). Both AR typing and PFGE have been used to characterise MRSA isolates recovered in Ireland since 1989 (Rossney and Keane, 2002; Rossney et al., 2003, 2004).

Between 1999 and 2003, MRSA isolated from blood cultures from human patients in hospitals which participate in EARSS have been typed ($n = 1580$

isolates from 1495 patients). This level of participation in EARSS provided a population cover rate of 95% in 2003. For ease of presentation, PFGE patterns were assigned 5-digit pulsed field type (PFT) numbers which were subsequently abbreviated to 2-digit PFT groups (PFG) (Rossney and Keane, 2002; Rossney et al., 2003, 2004). During the study period, the prevalence of one AR type-PFG, designated AR type AR06-PFG, PFG01, increased from 22% in 1999 to 80% in 2003. This strain is similar to the UK epidemic strain EMRSA-15 (Anon., 2000; Rossney et al., 2004).

This study reports the isolation of MRSA from 25 animals and 10 veterinary personnel. AR typing and PFGE analysis were used to investigate the relationship between the isolates from animals and humans and to elucidate whether human-to-animal or animal-to-human transmission might have occurred.

2. Materials and methods

2.1. Bacterial isolation and identification

As part of the diagnostic service provided for the University Veterinary Hospital (UVH) at University College Dublin (UCD) and for external veterinary practitioners, clinical specimens from infected animals are routinely processed for culture and susceptibility testing by the Diagnostic Veterinary Bacteriology Laboratory, UCD. During the course of the present study, in addition to clinical specimens, nasal swabs from available veterinary personnel in seven practices where MRSA was isolated, were screened for MRSA.

All specimens were inoculated onto the following culture media: Columbia Blood Agar Base (Oxoid Ltd., Basingstoke, UK; CM0331) supplemented with 5% defibrinated sheep blood (CBA), CBA with Staph/Strep Selective Supplement (Oxoid SR0070), MacConkey Agar No. 2 (Oxoid CM0109) and Purple Agar Base (BD Difco™, Sparks, MD, USA; 222810) supplemented with 1% (w/v) maltose. Staphylococcal isolates were identified by colonial morphology, Gram-stain, catalase, clumping factor and staphylococoagulase (tube coagulase) reactions (Quinn et al., 1994). Identification was confirmed with API kits, either API STAPH or ID 32 STAPH (bioMérieux SA, Marcy l'Etoile, France).

2.2. Susceptibility testing for clinical therapy

Antibiotic susceptibility for clinical therapy was determined by disc diffusion on Iso-Sensitest Agar (Oxoid CM0471) with an inoculum density equivalent to a 0.5 McFarland turbidity standard. Plates for susceptibility testing were incubated at 37 °C for 18 h. *S. aureus* ATCC 25923 was used as a quality control strain. Susceptibility to the following classes of antibiotics was determined [figures in parentheses indicate concentration (μg) per disc]: β-lactams [penicillin (10 units), amoxycillin/clavulanic acid (20/10), cephalothin (30)], lincosamines [clindamycin (2)], macrolides [erythromycin (15)], aminoglycosides [gentamicin (10)], fluoroquinolones [marbofloxacin (5), enrofloxacin (5)], tetracycline (30) and trimethoprim-sulphamethoxazole (1.25/23.75) (Quinn et al., 1994). Antibiotic discs were obtained from Oxoid Ltd. Methicillin resistance was detected on Iso-Sensitest Agar with 2% (w/v) sodium chloride and incubation at 30 °C using methicillin (5 μg/disc). The Mastalex™-MRSA kit (Mast Diagnostics, Merseyside, UK) was used to confirm phenotypic expression of methicillin resistance. The presence of the *mecA* gene was investigated in 22 isolates exhibiting phenotypic expression of methicillin resistance by a PCR assay described previously (Okuma et al., 2002).

2.3. Epidemiological typing

MRSA isolates were sent to the National MRSA Reference Laboratory (NMRSARL), St. James's Hospital, Dublin, for epidemiological typing. On receipt in NMRSARL, isolates were confirmed as MRSA by detecting staphylocoagulase (tube coagulase test) and methicillin resistance by disc diffusion testing at 30 °C using 10 μg methicillin disks (Oxoid Ltd.) on Columbia agar (LabM Lab1; International Diagnostics Group plc, Bury, UK) plates supplemented with 7% (w/v) horse blood (Rossney et al., 2003). Isolates were characterised by AR typing, biotyping and by PFGE analysis.

2.3.1. AR typing

AR typing was performed by determining susceptibility to a panel of 23 antimicrobials using a modified Stokes' disc diffusion technique on Diagnostic Sensitivity Test Agar (Oxoid CM0261) as described

previously (Rossney et al., 1994a,b, 2003; Rossney, 1995; Anon., 2000). Susceptibility to the following antimicrobial and chemical compounds was determined [figures in parentheses are the concentrations (μg/disc) used]: amikacin (30), ampicillin (10), cadmium nitrate (6), chloramphenicol (10), ciprofloxacin (1), erythromycin (5), ethidium bromide (20), fusidic acid (10), gentamicin (10), kanamycin (30), lincomycin (2), mercuric chloride (10), mupirocin (5 and 200), neomycin (30), phenyl mercuric acetate (10), rifampicin (2), spectinomycin (500), streptomycin (25), sulphafurazole (100), tetracycline (10), tobramycin (10), trimethoprim (1.25) and vancomycin (5). Most antibiotic discs were purchased from Oxoid Ltd. Five antimicrobials (spectinomycin, ethidium bromide, cadmium nitrate, phenyl mercuric acetate and mercuric chloride) were obtained from Abtek Biologicals Ltd. (Liverpool, UK). Isolates were assigned an AR type number on the basis of the susceptibility pattern produced. AR subtypes were determined using information obtained from full characterisation of collections of Dublin MRSA isolates during previous studies (Rossney et al., 1994a,b; Rossney, 1995). Isolates were not assigned an AR type (designated 'No Type', 'NT') if their AR profiles showed resistance to spectinomycin with otherwise susceptible patterns, or if they yielded susceptible patterns but were resistant to lincomycin because experience in NMRSARL had shown that isolates exhibiting these AR patterns required further characterisation by PFGE (Rossney et al., 2004). Biotypes (pigment production and ability to hydrolyse urea and Tween 80) were investigated as described previously (Coia et al., 1990).

2.3.2. PFGE typing

Total cellular DNA was extracted using achromopeptidase as described previously (MacKenzie et al., 2002). DNA was digested with *Sma*I (Promega R6121; Promega Corporation, Madison, WI, USA) and fragments were separated by PFGE using a CHEF DRIII PFGE apparatus (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK). Electrophoresis parameters were 6 V/cm with an angle of 120° and switch times of 6.8–63.8 s over 23 h at 14 °C. The software package GelCompar, Version 4.1 (Applied Maths, Sint-Martens-Latem, Belgium) was used to analyse banding patterns. Pattern differences were interpreted

as recommended by Tenover et al. (1995). *S. aureus* NCTC 8325 was included with each batch of isolates investigated.

2.4. Comparison of MRSA isolates from animals with MRSA from blood cultures from human patients

The Irish NMRSARL has databases of AR and PFGE typing patterns of MRSA recovered from: (1) blood cultures from human patients in Irish hospitals that participate in EARSS ($n = 1580$ isolates from 1495 patients between 1999 and 2003); (2) all specimen sites from patients in all hospitals collected during a 2-week study of MRSA in both the North and South of Ireland in 1999 ($n = 714$) and (3) isolates

representative of strains prevalent in one group of Dublin hospitals since the 1970s (Rossney et al., 1994a,b, 2003; Rossney, 1995; Rossney and Keane, 2002; Shore et al., 2005). For ease of comparison, PFGE patterns are assigned 5-digit pulsed field type (PFT) numbers which are then abbreviated to 2-digit PFT groups (PFG) following visual inspection of the gel images (Rossney et al., 2004).

In the present study, AR and PFGE typing patterns from MRSA from veterinary sources were compared with all patterns held in NMRSARL databases. A visual presentation of the comparison was prepared by drawing a dendrogram from representative PFGE patterns obtained with isolates in the present study compared with PFGE patterns representative of patterns obtained from MRSA isolates investigated

Table 1
Data relating to MRSA isolates from 17 animals and six attendant veterinary healthcare personnel

Veterinary practice, case, and specimen details						Antibiogram-resistogram (AR)			PFGE
Location	Practice	Case	Date	Species	Site	Ur[a]	Pattern	Type	Type (PFT[c])
Southwest	B	1	13 January 2003	Canine	Wound	−	ApCdCpErLnTe[b]	'NT'	A (01018)
Southwest	B	2	12 February 2003	Canine	Wound	−	ApCdCpErLn...	'NT'	A (01018)
Southwest	B	3	18 February 2003	Canine	Wound	−	ApCp...ErLn...	'NT'	A (01018)
Southwest	B	4	07 March 2003	Canine	Wound	−	ApCdCpErLnTe	'NT'	A (01018)
Southwest	B	5	17 April 2003	Canine	Wound	−	ApCdCpErLnTe	'NT'	A (01018)
Southwest	B	6	03 September 2003	Lapine	Wound	−	Ap...CpErLnTe	'NT'	A (01018)
Southwest	B	BH1	07 March 2003	Human	Nares	−	ApCdCpErLnTe	'NT'	A (01018)
West	C	7	16 April 2003	Canine	Nares	−	ApCdCpEr...	06.5	A (01018)
West	H	8	29 September 2003	Canine	Wound	−	ApCdCpEr...	06.5	A (01018)
Midlands	K	9	19 March 2004	Canine	Wound	−	ApCdCpEr...	06.5	A (01018)
Midlands	K	10	11 May 2004	Canine	Trach	−	ApCdCpErLn	'NT'	A (01018)
Midlands	K	KH1a	25 March 2004	Human	Nares	−	ApCdCp......	06.3	A (01018)
Midlands	K	KH1b	13 August 2004	Human	Nares	−	ApCdCpEr...	06.5	A (01018)
Midlands	K	KH2a	25 March 2004	Human	Nares	−	ApCdCpEr...	06.5	A (01018)
Midlands	K	KH2b	13 August 2004	Human	Nares	−	ApCdCpErLn	'NT'	A (01018)
Midlands	K	KH3	01 April 2004	Human	Nares	−	ApCdCpEr...	06.5	A (01018)
East	A	11	08 January 2003	Feline	Urine	−	ApCdCpEr...	06.5	A$_1$ (01002)
East	M	12	16 April 2004	Canine	Wound	−	ApCdCpEr...	06.5	A$_2$ (01075)
East	L	13	12 May 2004	Canine	Wound	−	ApCdCpErLn	'NT'	A (01018)
East	N	14	30 July 2004	Canine	Wound	−	ApCdCpErLn	'NT'	A (01018)
East	N	15	30 July 2004	Canine	Wound	−	ApCdCpErLn	'NT'	A (01018)
East	N	NH1	06 August 2004	Human	Nares	−	ApCdCpErLn	'NT'	A (01018)
East	N	NH2	06 August 2004	Human	Nares	−	ApCdCpErLn	'NT'	A (01018)
East	D	16	23 June 2003	Canine	Wound	−	ApCdCpErLn	'NT'	A (01018)
East	G[d]	17	17 September 2003	Phocine	LNS	−	ApCdCpEr...Fd	06.5FdR	A$_3$ (01074)

[a] Urease reaction.
[b] Antimicrobial abbreviations: Ap, ampicillin; Cd, cadmium nitrate; Cp, ciprofloxacin; Er, erythromycin; Fd, fusidic acid; Ln, lincomycin; Te, tetracycline.
[c] Other abbreviations: PFT, pulsed field type; BH1, KH1, NH1, isolates recovered from humans in veterinary Practices B, K and N, respectively. Repeat isolates were obtained from two humans (KH1 and KH2). Trach, tracheostomy tube; LNS, lymph node and spleen.
[d] Isolate was recovered post-mortem from a seal which had been treated in a seal sanctuary.

by the NMRSARL during the fourth quarter of 2003 ($n = 108$). DNA band patterns were analysed by the unweighted pair group method using arithmetic averages and similarity was calculated using the Dice coefficient. Pattern similarity was investigated at a threshold of 1% band matching tolerance. A cut-off value of 80% similarity was used to define a cluster (Murchan et al., 2003).

3. Results

3.1. Isolates of MRSA

The diagnostic laboratory of the UVH is one of a number of laboratories in Ireland and the UK which receive samples from veterinary practices in Ireland. Between January 2003 and October 2004, 133 isolates of *S. aureus* were obtained from approximately 3400 bacteriological samples processed by the diagnostic laboratory of the UVH. Of these isolates, 47 were MRSA, which were cultured from specimens from 25 animals and from nasal swabs from 10 attendant veterinary personnel. All isolates tested for the presence of *mecA* ($n = 22$) were positive. The animals comprised 14 dogs, eight horses, one cat, one rabbit and a seal from 16 different veterinary practices in nine counties. The 10 personnel were all healthy carriers who worked in one of four different practices or in the veterinary hospital. Details of 17 isolates from the non-equine animals and their attendant veterinary personnel ($n = 6$) are shown in Table 1. Thirteen of these 17 isolates (77%) were recovered from wounds. The remaining four isolates were from a tracheostomy tube from a dog, a urinary catheter from a cat, the nares of a dog and a lymph node and spleen from a seal. The seal was submitted for post-mortem examination following its death in a seal sanctuary where it had been held and treated for an unknown length of time. Details of the isolates from the eight horses and their attendant personnel ($n = 4$) are shown in Table 2. Seven of these isolates were recovered from wounds and one from an abdominal granuloma.

3.2. Susceptibility testing for clinical therapy

Despite occasional variation in results from in vitro susceptibility testing, MRSA isolates are invariably resistant to all β-lactam antibiotics. With one exception (Table 1), non-equine isolates were resistant to one or more of the following classes of antimicrobial agents: fluoroquinolones, lincosamines and/or tetracyclines. Of the non-equine isolates, only those from Practice B were tetracycline-resistant. No isolate exhibited resistance to more than three classes of antimicrobial agents. Unlike the non-equine isolates, MRSA from horses were resistant to aminoglycosides, tetracyclines and trimethoprim/sulphamethoxazole and were variably resistant to fluoroquinolones, lincosamines and rifampicin. One of the equine isolates (from Case 25), unlike the other seven isolates, was susceptible to trimethoprim (Table 2).

3.3. Epidemiological typing

3.3.1. Non-equine isolates

AR and PFGE typing confirmed that the 17 isolates from non-equine animals were unlike the isolates obtained from the eight horses (Tables 1 and 2). The majority of the former (64%, 11/17) exhibited an AR pattern designated 'NT' because the pattern exhibited resistance to tetracycline and/or lincomycin. The remaining six isolates exhibited the AR type, AR06. With three exceptions, a single PFGE pattern (Pattern A) was obtained from all 17 isolates. The patterns obtained from these three isolates differed by 4–6 bands from the other 14 isolates, which according to a strict application of the criteria of Tenover et al. (1995), suggests that these three isolates are 'possibly related' to them. The three exceptions included the isolate from the seal which was the only fusidic acid-resistant isolate investigated, the feline isolate and one isolate from a dog (Table 1). All 17 isolates were urease-negative.

Isolates from the six attendant veterinary personnel were indistinguishable from animal isolates in their respective practices. In Practice B, the single isolate recovered from a staff member exhibited an AR pattern which was indistinguishable from that obtained from three animals in the practice and had a PFGE pattern which was indistinguishable from all other isolates in that practice. This was the only isolate from any staff member that was tetracycline-resistant and Practice B was the only practice from which tetracycline-resistant isolates were recovered from small animals. Further details of the cases from this

Table 2
Data relating to MRSA isolates from eight horses and four attendant veterinary healthcare personnel

Veterinary practice, case and specimen details						Antibiogram-resistogram (AR)			PFGE
Location	Hospital/practice	Case	Date	Species	Site	Ur[a]	Pattern	Type	Type (PFT[c])
North	E/F[b]	18	04 September 2003	Equine	Wound	+	ApCdCpEbErGnKn...NmRfSuTbTeTp	Unf	B (00173)
Midlands	E/I	19	08 October 2003	Equine	Wound	+	ApCdCpEbErGnKn...NmRfSuTbTeTp	Unf	B (00173)
East	E/J	20	22 December 2003	Equine	Wound	+	ApCdCpEbErGnKnLnNmRfSuTbTeTp	Unf	B (00173)
North	E/F	21	26 July 2004	Equine	Wound	+	ApCdCpEbErGnKnLnNmRfSuTbTeTp	Unf	B (00173)
North	E/F	23	20 September 2004	Equine	Wound	+	Ap.........ErGnKn...Nm...SuTbTeTp	Unf	B (00173)
Midlands	E/O	22	20 September 2004	Equine	Wound	+	ApCd...EbErGnKnLnNmRfSuTbTeTp	Unf	B (00173)
East	E/P	24	23 September 2004	Equine	Wound	+	Ap.........ErGnKnLnNm...SuTbTeTp	Unf	B (00173)
West	E/Q	25	11 October 2004	Equine	Abdominal granuloma	+	Ap.........ErGnKn...St...SuTbTe...	Unf[d]	B₁ (00191)
North	F	FH1[e]	10 August 2004	Human	Nares	+	ApCd.....ErGnKn......RfSuTbTeTp	Unf	B₃ (00190)
North	F	FH2	10 August 2004	Human	Nares	+	ApCd.....ErGnKn......RfSuTbTeTp	Unf	B₃ (00190)
East	E	EH1	22 September 2004	Human	Nares	+	Ap ....ErGnKn......RfSuTbTeTp	Unf	B₂ (00189)
East	E	EH2	22 September 2004	Human	Nares	+	Ap.........ErGnKnLn...RfSuTbTeTp	Unf	B (00173)

[a] Urease reaction.

[b] Veterinary hospital and practice of origin.

[c] Abbreviations: PFT, pulsed field type; Ap, ampicillin; Cd, cadmium nitrate; Cp, ciprofloxacin; Er, erythromycin; Eb, ethidium bromide; Fd, fusidic acid; Gn, gentamicin;, Kn, kanamycin; Ln, lincomycin; Nm, neomycin; Rf, rifampicin; Su, sulphonamide; St, streptomycin; Tb, tobramycin; Te, tetracycline; Tp, trimethoprim; Unf, unfamiliar pattern.

[d] This pattern was unlike the patterns obtained from the other equine isolates.

[e] EH1, EH2, FH1, FH2, isolates recovered from humans in veterinary Practices E and F, respectively.

practice are reported elsewhere (Leonard et al., in press). In Practice K, five isolates from three staff members were typed. A second isolate from one staff member (after a 5-month interval) showed resistance to erythromycin, unlike the first isolate which was susceptible, while a second isolate from another staff member showed resistance to lincomycin. One isolate from each staff member was indistinguishable by AR typing from the isolates from the two animals in this practice. All isolates from both staff and animals were indistinguishable by PFGE. Two staff members from Practice N also yielded MRSA which was indistinguishable by AR typing from the isolates from animals in that practice. No human carriers were identified in association with other canine isolates.

3.3.2. Equine isolates

AR typing showed that isolates from horses were unlike the isolates from the other 17 animals and exhibited a more resistant AR pattern, showing resistance to up to 13 antimicrobial agents in the AR typing panel. These patterns were designated 'Unfamiliar' because they were unlike any patterns in NMRSARL's AR typing patterns database. The AR patterns of the four earliest isolates differed from each other with regard to lincomycin. Later isolates were susceptible to cadmium, ciprofloxacin, ethidium bromide and/or rifampicin. One isolate (Case No. 25) exhibited an AR pattern that differed with regard to seven antimicrobials from the earliest case suggesting that this isolate was not part of the original cluster. Unlike the non-equine isolates, all equine isolates were urease-positive.

A single PFGE pattern (Pattern B) was obtained from seven of the eight isolates from horses. This pattern differed by more than six bands from the PFGE patterns of the isolates from non-equine animals and hence these isolates were considered to be unrelated to the non-equine MRSA isolates. Case 25 exhibited a PFGE pattern (Pattern B_1) that differed by six bands from the remaining equine isolates. A strict application of the Tenover interpretative criteria suggests that this isolate is possibly related to the remaining equine isolates (Tenover et al., 1995).

The PFGE patterns of isolates obtained from veterinary personnel associated with the equine cases were different from the isolates from the humans associated with non-equine animals. One isolate from a staff member in Practice E yielded Pattern B. The PFGE pattern obtained from an isolate from a second staff member in that practice (Pattern B_2) differed by three bands from this pattern, suggesting that this isolate was closely related. The isolates from two staff members in Practice F yielded a pattern (Pattern B_3) that differed by seven bands from Pattern B but by three bands from Pattern B_1. Dendrogram analysis showed that Patterns B_1 and B_3 formed a sub-cluster that shared only 74% similarity with Pattern B.

All equine isolates were obtained from cases referred to a specialist veterinary hospital (Hospital E) and the typing data suggested that cross-infection may have occurred within that hospital and possibly in one referring practice (Table 2). The first equine isolate was obtained from a horse referred by Practice F in September 2003. Two further isolates were obtained from horses referred by Practices I and J in October 2003 and December 2003, respectively. No further cases were identified until July 2004 when an isolate was obtained from another horse referred by Practice F. Carriage among staff members was recognised in Practice F and Hospital E in August and September 2004, respectively. Two further infections occurred in September 2004 in Hospital E, in horses referred by Practices O and P. The source of the MRSA in Case 18, the index case, was not identified.

3.4. Comparison of MRSA isolates from animals with MRSA from blood cultures from human patients

Fig. 1 is a dendrogram drawn from the PFGE patterns obtained with the isolates in the present study and representative patterns obtained from isolates from human patients submitted to the NMRSARL during 2003. This comparison shows that the equine isolates (PFGE Pattern B; PFT 00173) are unlike the other animal isolates. PFGE Pattern A (non-equine animal isolates) is indistinguishable from PFT 01018 which is the most frequently occurring pattern among isolates recovered from human patients. Approximately 80% of MRSA from human patients investigated by NMRSARL during 2003 exhibited AR type AR06 (or variants of this AR type such as the 'NT' patterns) and 40% yielded PFT 01018 (Rossney et al., 2004). The PFGE patterns, A and B, formed two clusters showing 40% similarity. The equine isolates
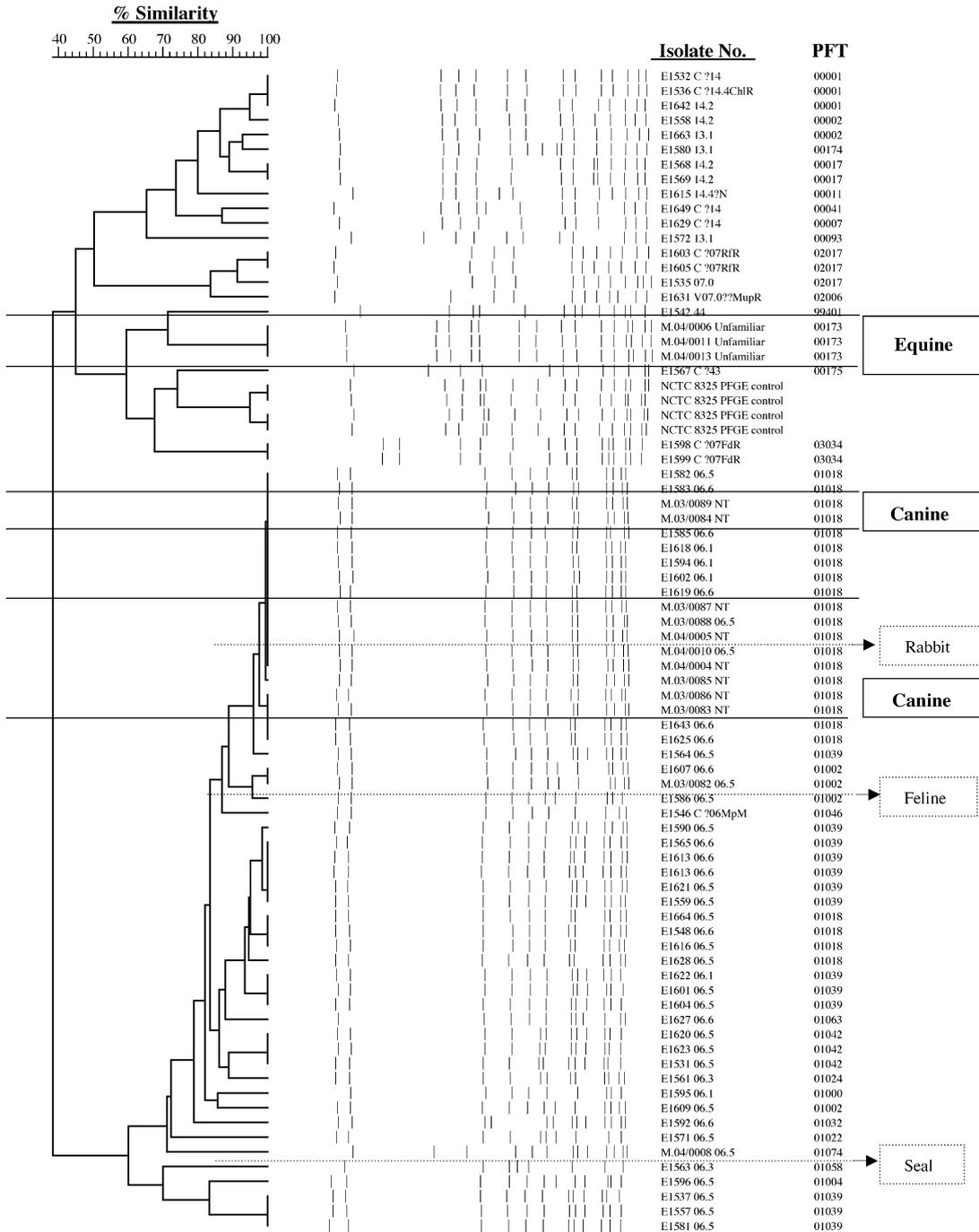
Fig. 1. Dendrogram (% similarity) drawn from DNA profiles representative of animal and human isolates with GelCompar using UPGMA; Dice coefficient; 1% tolerance; 0.5% optimisation and 0% minimum area. Representative profiles from animal isolates exhibiting PFGE types A (PFT 01018); A$_1$ (PFT 01002) and A$_3$ (PFT 01074) from non-equine animals and PFGE type B (PFT 00173) from horses are shown. Isolates carrying the prefix 'E' and 'M' were recovered from blood cultures from human patients and animals, respectively. The animal species from which the isolates were recovered are indicated. PFT, pulsed field type.

fell into Cluster 1 but showed only 71% similarity with the most closely related isolate from a human source and <60% similarity with any other isolates from humans. The canine isolates and the rabbit isolate shared an indistinguishable pattern with isolates exhibiting the AR pattern AR06 (PFT 01018). The feline isolate shared 89% similarity with this pattern while the seal isolate showed only 71% similarity.

4. Discussion

Although Ireland has a high rate of MRSA infection among human patients, until recently there have been no published reports of MRSA isolates from animals (Leonard et al., in press). In the present study, MRSA from non-equine animals resembled isolates of the predominant strain found in human patients in Ireland. This strain, which is similar to UK EMRSA-15, exhibits a limited number of AR and PFGE patterns making interpretation of epidemiological typing results difficult (Rossney et al., 2004). One of the assumptions underlying PFGE pattern interpretation is that epidemiologically unrelated isolates should have different genotypes (Tenover et al., 1995). This may not be the case with MRSA in Ireland because of the high prevalence of this one strain. Hence there is a problem trying to assess whether apparent clusters of isolates of this strain are occurring as a result of cross-infection or because of independent acquisition. It should be remembered that, although PFGE is considered the 'gold standard' for epidemiological typing of MRSA, it is a relatively insensitive typing method where differences rather than similarities are conclusive. Previous studies of MRSA in Ireland have shown that other typing methods (for example, AR typing) can differentiate isolates that are indistinguishable by PFGE (Rossney et al., 2003).

In the present study, tetracycline resistance was a useful marker to support the epidemiological evidence of an outbreak in Practice B but whether the two tetracycline-susceptible isolates from that practice were part of the same outbreak is uncertain. An investigation of the animals' owners for MRSA carriage might reveal a different source for these two isolates. Antimicrobial use in both animals and humans also needs to be ascertained because it is an important factor that needs to be considered when interpreting AR typing results. The data relating to clusters from the other practices must also be interpreted with caution, because of the prevalence of this strain in human hospitals in Ireland. While these clusters may represent transmission within the practices, they too, may have been acquired from other sources. For example, their owners may have had human healthcare-associated risk factors facilitating acquisition of MRSA.

The phocine isolate illustrates the dilemma of an isolate with a PFGE pattern that should be interpreted as 'possibly related' but, in the absence of other evidence, is unlikely to be epidemiologically related. This MRSA was the only isolate that was resistant to fusidic acid. Resistance to fusidic acid should also be interpreted with caution because of the ease with which resistance occurs. However, if the patient was not treated with this antibiotic, it is likely that this isolate is unrelated to the rest of the cluster.

The equine isolates are unlike MRSA seen in human medical practice in Ireland and seven of them exhibit indistinguishable PFGE patterns. MRSA isolates from horses were first noted in Canada in 2000 where it was reported that the infecting strain originated from but was relatively uncommon in humans (Weese, 2004). That strain designated in Canada as Canadian epidemic MRSA 5 (CMRSA-5) subsequently adapted to the equine environment and by 2004, was spreading within the equine population.

Epidemiological typing of the isolates from horses, together with the epidemiological data, strongly suggest that two outbreaks of infection occurred. No source was identified for the index case (Case 18), which initiated an outbreak in Hospital E in 2003. The evidence suggests that a concurrent outbreak persisted in Practice F during this time and that the strain was re-introduced into Hospital E in July and September 2004. The latter isolate had lost resistance to cadmium, ciprofloxacin, ethidium bromide and rifampicin but was indistinguishable by PFGE from the earlier isolates. Isolates from staff in Practice F and Hospital E in August and September 2004 exhibited the more susceptible AR pattern but were susceptible to neomycin also. One of these isolates exhibited PFGE Pattern B and one a closely related pattern (B_2), but the pattern from isolates from the two staff members from Practice F differed by seven bands from Pattern B. Applying a strict interpretation of the criteria of

Tenover et al. (1995), these isolates could be considered unrelated. However, in view of the variation in AR pattern that the strain was demonstrating, it is likely they too represent the outbreak strain. It is tempting to speculate that, like the Canadian strain, this strain originated from a human hospital and that removal from the antibiotic pressure in the human hospital environment has resulted in loss of resistance to these antimicrobial agents (Weese, 2004).

Typing results from the eighth equine isolate were difficult to interpret. The PFGE profile suggested that this isolate was possibly related to the other equine isolates but the AR pattern included resistance to streptomycin and susceptibility to trimethoprim. Thus it differed by seven characters from the first equine isolate and may be more distantly related than the PFGE results suggest. Multilocus sequence typing and SCC*mec* typing will be undertaken to complete the molecular characterisation of these isolates.

In the present study, no attempt was made to investigate carriage of MRSA by owners or by uninfected animals. Weese et al. (2004b) have shown that asymptomatic colonisation in horses is a serious problem with 4.7% (46/972) of horses colonised. Data in the current report were obtained from one veterinary hospital and from veterinary practices that refer cases to that hospital or specimens to its associated diagnostic microbiology laboratory. There are no published reports of MRSA from other veterinary practices and veterinary hospitals in Ireland but anecdotal evidence suggests that MRSA isolates are being recovered from some of these practices and hospitals also.

The significance of nasal carriage of MRSA among veterinary personnel is undetermined. In the human population, infected and colonised patients are the main reservoirs of MRSA with transmission occurring mainly via the transiently colonised hands of healthcare workers. When dealing with a strain of MRSA that is prevalent in the human population, it is important to consider whether the veterinary personnel themselves have human healthcare-associated risk factors for the acquisition of MRSA.

The emergence of MRSA in animals and veterinary personnel in Ireland presents a range of problems not least of which is the fact that the human-associated strain reported in the present study is notoriously difficult to control and eradicate if it becomes established in institutions such as hospitals or nursing homes. Most guidelines stress that aggressive infection control measures can be successful if applied when MRSA is first recognised in an institution. If MRSA becomes endemic, the problem is extremely difficult to control. Guidelines for the control of MRSA in animals need to be formulated as a matter of urgency to minimise future problems with this organism. A recent review presents a constructive approach to the control of MRSA in equine hospitals and stables and could provide a useful framework for the control of MRSA in animals in Ireland (Weese, 2004).

5. Conclusion

This paper documents the isolation of MRSA from clinical specimens in several animal species in Ireland, with the recovery of similar isolates from veterinary personnel associated with the care of these animals. Isolates were divided into two strains by AR and PFGE typing. One strain was associated with small animals and was indistinguishable from the most common strain seen in human hospitals in Ireland. The other strain was associated with horses and equine veterinary personnel and has not been reported previously in Irish human patients. The results suggest that transmission between animals and humans can be of both veterinary and public health importance. Veterinary personnel need to be aware of the possibility of MRSA infection in animals and their possible role in transmission of this organism to animals. Infection control procedures for MRSA in veterinary practices are urgently required to minimise transmission of this organism. In addition, veterinary practitioners have a duty to provide appropriate advice to owners of MRSA-infected animals.

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