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spa typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland

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***Objectives*: Region X of the protein A gene (*spa*) was sequenced from methicillin-resistant *Staphylococcus aureus* (MRSA) isolates originating from animals, humans and the environment at veterinary hospitals in the UK and Ireland. MRSA transmission between animals and veterinary staff was assessed on the basis of *spa* typing, PFGE and epidemiological data.**

***Methods*: MRSA isolates from dogs ($n = 27$), horses ($n = 9$), cats ($n = 6$), staff ($n = 22$) and environmental surfaces ($n = 3$) were analysed by PFGE and *spa* typing. Known contacts between human and animal MRSA carriers were ascertained from the veterinary hospitals.**

***Results*: All feline, most canine (96%) and human (82%) isolates showed PFGE profiles that were either indistinguishable (subtype A1) or closely related (subtypes A2–A10) to that of the epidemic clone EMRSA-15 (CC22), whereas most equine isolates (88%) were related to CC8 (types C, D, E and G). *spa* polymorphism enabled discrimination among MRSA strains assigned to the same PFGE type. Fifteen *spa* types clustering into two distinct groups were detected, with t032 being the most prevalent (48%). The *spa* and PFGE types of MRSA isolated from seven staff members were the same as those of strains isolated from infected animals attended by the staff.**

***Conclusions*: Irrespective of geographical origin, MRSA isolated from equine and small animal hospitals generally clustered into two distinct clonal complexes, CC8 and CC22, respectively. The combined use of *spa* and PFGE typing allowed better discrimination than each method used individually, and provided useful information on MRSA transmission between animal and human individuals.**

Keywords: MRSA, typing, dogs, horses, veterinarians

Introduction

Methicillin was first introduced in human clinical practice in 1959 for the treatment of infections caused by penicillin-resistant *Staphylococcus aureus*. The first methicillin-resistant *S. aureus* (MRSA) strain was reported in 1961 in the UK.[1] Since then, the incidence of MRSA infections has been increasing steadily. Today, MRSA is one of the most important nosocomial pathogens worldwide and a serious public health concern as infections cause high morbidity and mortality.[2,3] In England, the number of death certificates reporting MRSA infection increased from 49 in 1993 to 890 in 2003 (http://www.statistics.gov.uk). MRSA is not confined to health care settings and has been reported increasingly as a cause of infection in healthy individuals, often in the absence of recognizable risk factors.[4] In most cases, clones causing community-acquired MRSA (CA-MRSA) infections, mainly skin

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and soft-tissue infections, differ genetically from those causing hospital outbreaks, are less resistant to other classes of antimicrobials and produce the Panton–Valentine leucocidin (PVL) toxin.[4]

In recent years, increasing numbers of reports have documented the occurrence of MRSA in companion animals, especially in dogs[5–11] and horses.[12–14] As in humans, MRSA can colonize skin, nasal and oral mucosae in healthy animals, and infections appear to be associated with risk factors such as hospitalization, surgery, wounds, chronic disease or immunosuppression. Various case reports have documented the occurrence of MRSA clones common in human infections, in companion animals and people in close contact (e.g. owners and veterinarians).[12–18] In 2005, three independent studies conducted in the UK[5,16] and in Ireland[18] revealed the occurrence of the epidemic clone EMRSA-15 in companion animals and veterinary staff. It has been estimated that EMRSA-15, belonging to clonal complex 22 (CC22), is the predominant epidemic clone in the UK, causing ~60% of MRSA bacteraemia cases in this country.[19]

Various techniques can be used for typing MRSA isolates.[20] PFGE is a highly discriminatory method and is generally considered as the 'gold standard' for MRSA outbreak investigations. However, it is laborious and inter-laboratory comparison of results is difficult.[21] Among the various DNA sequence-based methods that have been developed to overcome these limitations, *spa* typing has been shown to be an effective and rapid method for typing MRSA.[22] The method is based on the number of tandem repeats and the sequence variation in region X of the protein A gene.[23] In this study, we investigated the possible use of *spa* typing in combination with PFGE for tracking MRSA transmission between animals and veterinary staff.

Materials and methods

Bacterial strains

The 67 MRSA strains analysed in this study were isolated at Queen Mother Hospital for Animals (QMHA) at the Royal Veterinary College in London, University of Liverpool's Small Animal Hospital (SAH), Philip Leverhulme Equine Hospital (PLEH) in Liverpool and University Veterinary Hospital (UVH) at University College Dublin (Table 1). The strain collection included 27 canine, 6 feline, 9 equine, 22 human and 3 environmental isolates. Strains collected at QMHA included strains that were isolated on a single day from 3 environmental sites and the nasal cavities of 13 staff members and 5 dogs,[16] and 17 diagnostic submissions (this study). At SAH strains

Table 1. Frequencies of *spa* and PFGE type combinations among 67 MRSA isolates from distinct veterinary hospitals and sources

	Veterinary hospital[a]				Isolation source					
PFGE/*spa* type combination	QMHA	SAH	PLEH	UVH	dog	cat	horse	staff	other[b]	Total isolates
A1, t020		1	1				1	1		2
A1, t022	6				2	1		3		6
A1, t025	1				1					1
A1, t032	10	4		6	12	2		6		20
A1, t628	2				1	1				2
A1, t749				1				1		1
A1, t883	5				1			1	3	5
A1, t1021		1			1					1
A1, t1041		1			1					1
A2, t032	2				1			1		2
A3, t032	2				1			1		2
A4, t032	1				1					1
A5, t020	1				1					1
A5, t032	3				1			2		3
A6, t379	1					1				1
A7, t032	1							1		1
A8, t032	1							1		1
A9, t032	1					1				1
A10, t032		1			1					1
A10, t1041		1			1					1
B1, t008	1							1		1
B2, t064				2				2		2
C, t036			3				3			3
D, t064				2			1	1		2
E, t451				3			3			3
F, t1042		1			1					1
G, t036			1				1			1

[a]QMHA, Queen Mother Hospital for Animals, Royal Veterinary College, London; SAH, University of Liverpool's Small Animal Hospital; PLEH, Philip Leverhulme Equine Hospital, Liverpool; and UVH, University Veterinary Hospital, University College Dublin.
[b]Hospital environment.

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were isolated over a 17 month period from the nasal cavities of 3 staff members and from 7 infected dogs as well as from 5 horses hospitalized at PLEH.[5] At UVH in Dublin, human strains were obtained through screening of veterinary staff working in seven practices where MRSA was previously isolated from diagnostic specimens.[18]

PFGE analysis

PFGE was done as previously described by Murchan *et al*.[24] Briefly, *S. aureus* colonies from overnight cultures were incorporated into agarose plugs. After bacterial lysis with lysozyme (100 mg/L), lysostaphin (10 000 U/mL) and RNase (50 mg/L) for 2 h at 37°C, genomic DNA was digested using *Sma*I (40 U/mL) at room temperature, overnight. PFGE was performed by clamped homogeneous electric field (CHEF) electrophoresis with a CHEF-DR III System (Bio-Rad Laboratories, CA, USA) in a 1.1% (w/v) gel according to the HARMONY protocol.[24] *S. aureus* NCTC 8325 was included as an internal control strain to normalize band distances among gels. Fragments ranging from 50 to 1000 kb were included in the analysis. Gels were analysed using BioNumerics v. 4.5 software (Applied Maths, Kortrijk, Belgium), and cluster analysis was performed by UPGMA based on the Dice similarity coefficient, with optimization and position tolerance set at 1.0%. MRSA isolates were clustered using an 80% homology cut-off, above which strains were considered to be closely related[24] and assigned to the same PFGE type. A reference strain of EMRSA-15 (PM-62) was included in the analysis and its PFGE pattern designated as type A1.

MLST and spa typing

Amplification of the *spa* repeat region was performed using primers spa-1113f (5′-AAAGACGATCCTTCGGTGAGC-3′) and spa-1514r (5′-CAGCAGTAGTGCCGTTTGCTT-3′) as previously described.[25] PCR products were sequenced using BigDye v1.1 (Applied Biosystems, Foster City, USA). The *spa* types were determined using the Ridom StaphType 1.4.1 software,[25] and clustered in *spa*-derived clonal complexes (*spa*-CC) by the Based Upon Repeat Pattern (BURP) algorithm using a cost setting of nine or less for allowed clustering.[26] An outlier was assigned to CC8 based on *spa* typing and its PFGE pattern (type G) similarity to previously typed isolates in the Danish MRSA reference laboratory strain collection. MLST was performed on a single isolate (PFGE and *spa* type combination; F, t1042) according to Enright *et al*.[27]

Collection and analysis of epidemiological data

Information on known contact that had occurred between animals and staff was provided by the four veterinary hospitals involved in the study. At QMHA, all personal data were handled confidentially according to the procedures approved by the Royal Veterinary College Ethics and Welfare Committee; MRSA-carrier staff were asked for permission to link their swab codes to MRSA-positive animals handled by them. At SAH, PLEH and UVH, human samples were obtained on a voluntary, informed consent basis and personal information kept strictly anonymous.

Results and discussion

Seven distinct PFGE types were observed among the 67 MRSA strains tested: type A relating to EMRSA-15 (CC22), types B, C, D, E and G relating to CC8, and type F relating to CC72. PFGE types A and B were subdivided into 10 (A1–A10) and 2 (B1–B2) subtypes, respectively, based on minor band variations. All feline and most canine (96%) and human (82%) isolates showed PFGE profiles that were either indistinguishable (type A1) or closely related (types A2–A10) to that of the epidemic clone EMRSA-15 (Figure 1), according to the Tenover criteria.[28] Eight of the nine equine isolates (88%) were related to CC8 (PFGE types C, D, E and G). The remaining equine isolate was related to EMRSA-15 (Table 1). To the best of our knowledge, this is the first report of EMRSA-15 in a horse in Europe. Apart from this exception, all MRSA occurring in horses and equine practitioners (CC8) were genetically different from those isolated from small animal clinics (CC22), thus providing evidence of two distinct genetic lineages occurring in the two different animal populations.

Sequence analysis of region X of the *spa* gene resulted in the detection of 15 distinct *spa* types, including two novel types (t1041 and t1042). The most frequent *spa* type was t032 (32/67 = 48%, Table 2) and the remaining *spa* types were most often associated with the geographical origin of the isolates (Table 1). Similar results were demonstrated by a previous study in Germany,[29] where all MRSA isolates from pet animals had *spa* type t032 (n = 16, 100%) and closely resembled hospital-associated MRSA clones. BURP analysis of the 15 *spa* types identified two distinct *spa* clusters associated with CC8 and CC22, respectively, and a novel singleton *spa* type, t1042 (Figure 2). By MLST typing, the t1042 isolate was identified as an ST72 (allelic profile: 1-4-1-8-4-4-3). Comparison between the assigned *spa*-clonal complexes (*spa*-CC) and PFGE types showed complete congruence. The same *spa* types were observed in various isolates from epidemiologically related individuals, such as dogs and their attendant veterinarians or veterinarians working at the same clinic. Seven cases of human nasal carriage were associated with exposure to MRSA-positive animals, mainly dogs with infected wounds prior to contact with the veterinary staff. MRSA infection through contact with veterinarians could be excluded in these cases, and transmission was more likely to have occurred from the dogs to the humans. Some of the most significant associations are explained in the following paragraphs.

QMHA, London

Two cases were noted where MRSA strains sharing the same PFGE and *spa* types were isolated from clinicians that had known direct contact with animal MRSA carriers. One clinician carried a strain having an identical PFGE (A5) and *spa* (t032) type to that originating from a dog with an infected wound that was attended to by the clinician. EMRSA-15, which is represented in this study by PFGE and *spa* type combination A1, t032, was isolated from a veterinarian and three dogs treated by the clinician. Two additional MRSA-positive dogs treated by this veterinarian were found to carry strains with distinct genotypes (A1, t022 and A4, t032; respectively). A third interesting association was noted between a human, a canine and three environmental strains. In this case, the genotype involved (A1, t883) had a different *spa* type compared with EMRSA-15 and the clinician had no known direct contact with the colonized dog. The recovery of this genotype from environmental sites (door handles in a consulting room and the male clients' toilet, and a marker pen on the ultrasound booking board) suggests that

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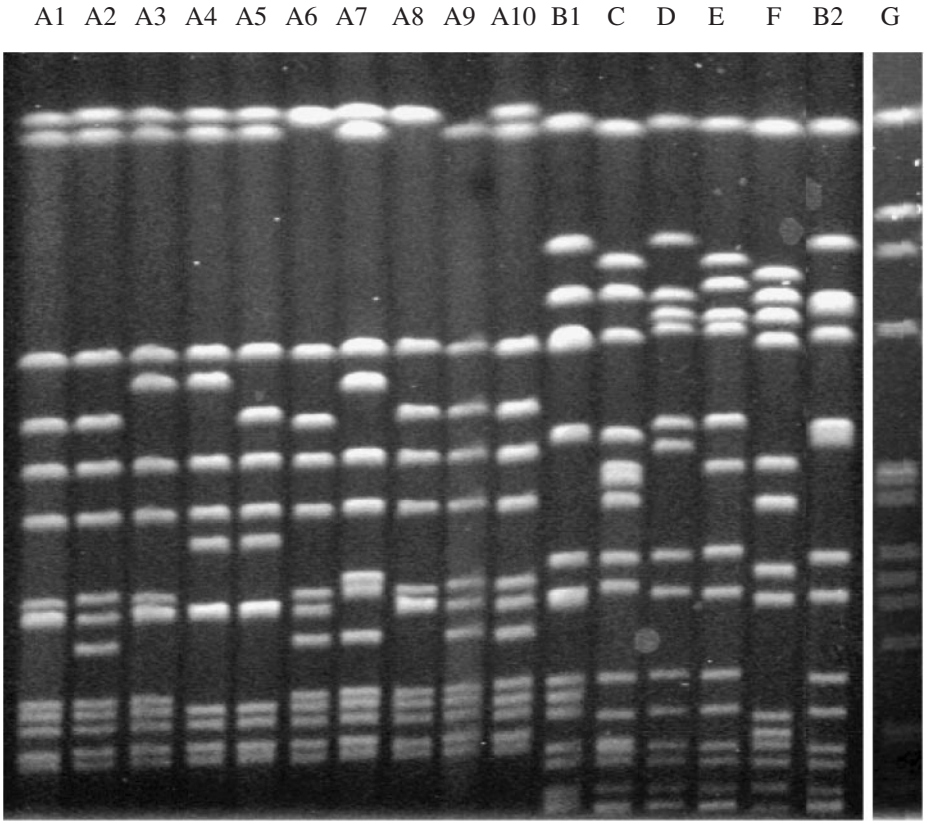

Figure 1. PFGE profiles (A, B, C, D, E, F and G) and subtypes (A1–A10 and B1–B2) observed among MRSA isolated from domestic animals and veterinary staff.

Table 2. Repeat successions, associations with PFGE subtypes, frequencies and predicted clonal complexes of the *spa* types detected in this study

spa type	Repeat succession	Associations with PFGE subtypes	Number of isolates *n*= 67	Predicted clonal complex
t008	11-19-12-21-17-34-24-34-22-25	B1	1	CC8
t020	26-23-31-29-17-31-29-17-25-17-25-16-28	A1, A5	3	CC22
t022	26-23-13-23-31-29-17-31-29-17-25-17-25-16-28	A1	6	CC22
t025	26-23-23-13-23-29-17-31-29-17-25-17-25-16-28	A1	1	CC22
t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	A1, A2, A3, A4, A5, A7, A8, A9, A10	32	CC22
t036	11-12-21-17-34-24-34-22-33-25	C, G	4	CC8
t064	11-19-12-05-17-34-24-34-22-25	B2, D	4	CC8
t379	26-23-23-13-23-31-29-17-25-17-25-16-28	A6	1	CC22
t451	11-12-05-17-34-24-34-22-25	E	3	CC8
t628	26-23-23-13-23-31-29-17-31-29-17-31-29-17-25-17-25-16-28	A1	2	CC22
t749	26-23-23-13-23-31-29-17-31-29-17-25-17-25-17-25-16-28	A1	1	CC22
t883	26-23-17-25-16-28	A1	5	CC22
t1021	26-23-23-13-23-31-29-17-31-29-17-25-17-25	A1	1	CC22
t1041[a]	26-23-23-13-23-31-29-17-28	A1, A10	2	CC22
t1042[a]	15-12-20-17-12-17	F	1	CC72

[a]Novel *spa* type.

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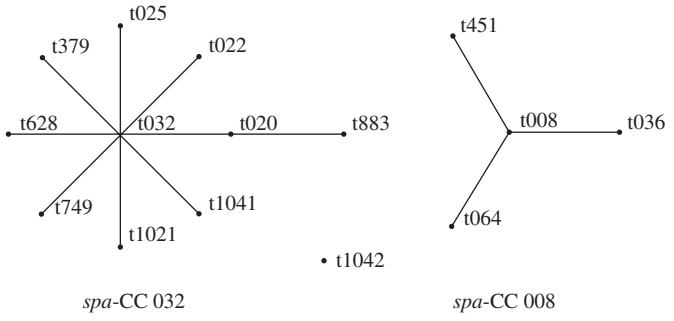

Figure 2. BURP analysis of the identified *spa* types showing two clusters (*spa*-CC 032 and *spa*-CC 008) and a singleton *spa* type (t1042).

environmental contamination could play an important role in MRSA dissemination within veterinary hospitals. The three animals that had been directly exposed to this clinician showed the same PFGE profile (A1) but a different *spa* type (t032). The remaining MRSA-positive human had not been exposed to known infected or colonized animals.

SAH, Liverpool

Indistinguishable MRSA strains corresponding to the EMRSA-15 genotype (A1, t032) were isolated from a veterinarian, a nurse and a dog with a joint infection attended by these personnel. A third human isolate from this hospital (strain 46) was obtained from a veterinary student who had worked with the clinician and the nurse on the above-mentioned dog. This isolate had an identical PFGE type but a different t020 *spa* type (Table 2), without apparent direct connection to any other animals. The student was sampled at a later date than the two veterinary personnel and it is plausible that this strain was obtained from another unidentified source. Four of the five equine MRSA isolates originating from PLEH had the same *spa* type (t036) but two distinct PFGE profiles (C and G), and the remaining isolate was related to EMRSA-15 (A1, t020). All equine strains obtained from PLEH were unrelated to those isolated from horses in Ireland.

UVH, Dublin

MRSA strains collected at University College Dublin were isolated from samples submitted from four geographical areas (Northern, Eastern, Western and South Western parts of Ireland). The strains originating from South Western and Eastern small animal practices were isolated from four dogs with infected wounds and from three veterinarians in direct contact with these dogs. With the exception of one strain (A1, t749), all canine and human strains were genetically related to EMRSA-15 (A1, t032) and were the same as those isolated from small animal hospitals in the UK. A veterinarian from Western Ireland and a horse on which he had operated shared a common PFGE pattern (D) and *spa* (t064) type. Two human isolates from a Northern practice had the same PFGE (B2) and *spa* (t064) types, which did not correspond to that of the three isolates (E, t451) from horses in Northern Ireland and thus no evidence of transmission between the veterinarians and the horses was found in this case. All of the horses came from the same premises but the veterinarians belonged to a large equine practice and would have had contact with many different horses on a daily basis. The *spa* type (t064) found in equine practitioners from Northern and Western practices differed from the prevalent type (t032) among veterinarians attending small animal hospitals situated in the Eastern and South Western parts of Ireland.

The polymorphic region X of the *spa* gene consists of a variable number of tandem repeats, generally 24 bp but repeats of 21 and 27 bp in length have been identified. The sequence variation in this region arises from duplications or deletions of repeats or point mutations within the repeat sequence.[30] Shopsin *et al.*[22] noted that the mutational rate in this region is relatively low, allowing for adequate discrimination between MRSA strains. Kahl *et al.*[31] studied the mutational rate within region X in 10 cystic fibrosis (CF) patients with persistent colonization by a single *S. aureus* clone, as determined by PFGE. The researchers calculated that one genetic event would occur every 70 months, and documented deletions as being the most common. A similar study also noted the above observations in *Haemophilus influenzae* isogenic strains isolated during persistent airway colonization of CF patients.[32]

The number of repeats within the *spa* gene varied between 6 and 19 among the 15 *spa* types observed in this study (Table 2). EMRSA-15 and related subtypes (PFGE type A) were characterized by a cluster of 10 *spa* types, including 1 novel type (t1041). However, the different *spa* types showed an overall similar composition and organization of repeats. The dominant *spa* type observed was t032 and consisted of 16 repeats. The number of different *spa* types associated with PFGE type A could be explained by possible microevolution of t032, which could lead to the development of the other observed *spa* types (Figure 2). The occurrence of a few genetic events within t032, deletions or duplications of repeats, would give rise to the other linked *spa* types as seen on Figure 2. MRSA strains of PFGE type B included two *spa* types that clustered together on the basis of BURP analysis (Figure 2).

This study demonstrates that *spa* typing and PFGE can be usefully combined to assess possible MRSA transmission between animals and veterinary staff. Large-scale epidemiological investigations of this type are urgently needed to evaluate whether veterinary staff are at risk for MRSA carriage. The emergence of MRSA in veterinary medicine raises important questions relating to both human and animal health. It is necessary to understand why MRSA have emerged in companion animals and to what extent animal carriers represent a risk to people in daily contact with them, such as veterinary staff, farm personnel and pet or horse owners. Dogs with infected wounds represent a potential MRSA reservoir for transmission to humans, and particular care should be taken when in contact with such animals. Similarly, human MRSA carriers pose a risk to domestic animals since transmission can occur in both directions.[33]

The results suggest that *spa* typing may be a useful tool for investigating MRSA transmission between individuals and spread within hospitals and between geographical areas. Although PFGE is generally regarded as the most discriminatory method for MRSA typing, *spa* typing enabled discrimination between isolates having the same PFGE profiles. Accordingly, *spa* typing should be considered as a convenient method in terms of speed, ease of use, interpretation and standardization, but also as a complementary tool providing added epidemiological value to PFGE typing.

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Transparency declarations

None to declare.

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