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Detection of three distinct genetic lineages in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from animals and veterinary personnel

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SUMMARY

This study involved the phenotypic and molecular characterization of a population of methicillin-resistant *Staphylococcus aureus* isolates from animals and from veterinary personnel in Ireland. Isolates from 77 animals (dogs, $n=44$; cats, $n=4$; horses, $n=29$) and from 28 veterinary personnel were characterized using their antimicrobial resistance profiles and pulsed-field gel electrophoresis patterns. In addition, a representative number of these isolates ($n=52$) were further analysed using *spa*-typing techniques. The results obtained identified the presence of three distinct clonal complexes, CC5, CC8 and CC22, in both animal and human isolates. Two of these clonal complexes, CC8 and CC22, respectively, have been previously described in animals in Ireland but the presence of the third complex CC5 is a novel finding. The significance of this development, in relation to human and animal healthcare, is discussed.

Key words: Antibiotic resistance, bacterial typing, methicillin-resistant *S. aureus* (MRSA), *Staphylococcus aureus*.

INTRODUCTION

Reports of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated infections in animals have been documented worldwide, affecting a diverse range of species including pets, food-producing animals and wild animals [1–3]. Research has shown that MRSA infections in pets have involved strains resembling human nosocomial strains, including epidemic MRSA (EMRSA) [4] and that acquisition was likely to have resulted from strains carried by human owners being passed to their animals [5]. Transmission of MRSA from animals to humans has also been documented [6]. In addition, a recent study in The Netherlands, reported a MRSA strain from a livestock animal reservoir entering the human population and this strain, clonal complex CC398, now accounts for over 20% of the strains isolated in that country [7].

Epidemiological typing of MRSA isolates using antimicrobial resistance, PFGE and *spa*-typing has proven useful in investigations of outbreaks of MRSA infections [8–10].

The characterization of a collection of MRSA isolates, associated with infection and isolates from colonized healthy animals and humans in Ireland is described. The likely sources for their acquisition and potential for transmission is discussed.

MATERIALS AND METHODS

The Diagnostic Veterinary Bacteriology Laboratory (DVBL) provides a diagnostic clinical service for the

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University College Dublin Veterinary Teaching Hospital (UVTH) and for private veterinary practitioners around Ireland. The isolates described in this study were obtained from clinical specimens from 3866 animals (dogs, $n=2864$; cats, $n=619$; horses, $n=383$) processed for routine microbiological analysis, and from specimens from 816 animals involved in prospective surveillance studies of both clinically affected (dogs, $n=143$; cats, $n=39$; horses, $n=65$) and clinically healthy (dogs, $n=286$; cats, $n=47$; horses, $n=236$) animals attending private clinics and a tertiary referral clinic [11]. Seventy isolates were from clinically affected animals (dogs, $n=43$; cats, $n=4$; horses, $n=23$) while seven were from asymptomatic carrier animals (dogs, $n=1$; horses, $n=6$). A further 28 isolates were obtained from nasal swabs from veterinary personnel following investigations into clinics with multiple MRSA cases. The animals and the veterinary staff, described in this study, resided in 19 of the 32 counties in Ireland. Only one isolate per patient was included in this study. The University College Dublin Animal Research and Ethics Committee approved this investigation.

Laboratory study

All isolates were confirmed as *S. aureus* by routine methodologies including colony morphology, Gram-stain, catalase, slide coagulase, latex agglutination test (Pastorex® Staph-Plus, Bio-Rad, France) and ability to ferment maltose in purple agar base (BD Difco™, USA) supplemented with 1% (w/v) maltose [12]. All coagulase-positive staphylococci were identified to species level using API Staph™ or API Rapid ID 32 Staph™ kits (bioMérieux France). Methicillin resistance was detected by determining resistance to cefoxitin (30 μg; Oxoid Ltd, UK) using the Clinical and Laboratory Standards Institute (CLSI) disc diffusion method [13]. Methicillin resistance was confirmed by detection of penicillin binding protein 2a using the Mastalex™ MRSA kit (Mast Diagnostics, UK). Additional antimicrobial susceptibility testing [antibiogram-resistogram (AR) typing] was performed on confirmed MRSA isolates against the following range of antimicrobial agents (disc concentrations given in parentheses): amikacin (30 μg), kanamycin (30 μg), neomycin (30 μg), streptomycin (25 μg), tobramycin (10 μg), ampicillin (10 μg), erythromycin (15 μg), lincomycin (2 μg), fusidic acid (10 μg), vancomycin (30 μg), rifampicin (5 μg), ciprofloxacin (5 μg), chlorampenicol (30 μg), mupirocin (5 μg), mupirocin (200 μg), sulphonamide (300 μg), trimethoprim (5 μg) (Oxoid Ltd), as well as to spectinomycin (500 μg), ethidium bromide (60 μg), cadmium acetate (130 μg), mercuric chloride (10 μg) and phenyl mercuric acetate (10 μg) (Abtek Biologicals, UK). The AR results of the 105 isolates in this study were compared to the Irish National MRSA Reference Laboratory (NMRSARL) database of profiles and assigned an AR number depending on the pattern produced [8]. Isolates were not assigned an AR type (designated 'No Type', NT) if they showed resistance to spectinomycin, lincomycin, streptomycin or tetracycline as such isolates required pulsed-field gel electrophoresis (PFGE) for correct classification [8].

PFGE was performed on all isolates, as previously described [14]. Briefly, total DNA was extracted using achromopeptidase and the DNA was then digested with *Sma*I (Promega R6121; Promega Corporation, USA) and fragments were separated using a CHEF DRIII PFGE apparatus (Bio-Rad Laboratories Ltd, 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. Banding patterns were analysed using the software package, GelCompar version 4.1 (Applied Maths, Belgium), with pattern similarity investigated at a threshold of 1·5% band-matching tolerance. Isolates were clustered using 80% homology cut-off, above which strains were considered to be closely related and assigned to the same PFGE type [9]. Final interpretation required visual analysis [15].

A number of isolates ($n=52$) were further characterized by *spa*-typing according to the *spa*-typing protocol as recommended by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet; www.seqnet.org/). These isolates were selected on the basis of their involvement in a cluster of cases within a practice or because they exhibited an unusual AR or PFGE pattern. Thirteen of these isolates had been previously characterized [16]. *Spa* types were determined using Ridom StaphType software version 1.4 (Ridom GmbH, Germany), and analysed by the implemented algorithm BURP which groups *spa* types by means of their relatedness to each other and to a common founder [17]. *Spa* types were part of the same clonal complex if the cost (reflecting the evolutionary distance between two isolates) was $\leqslant 6$.

RESULTS

Characterization by *spa*-typing, PFGE and AR typing demonstrated that the MRSA strains described in

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this study belonged to one of three distinct MRSA lineages, clonal complexes CC5, CC8 and CC22 (Table 1). No association was apparent between the clinical origin of an isolate and the clonal complex to which it belonged; strains from each lineage were isolated from both clinically affected and healthy animals.

All isolates demonstrated resistance to all of the β-lactam antibiotics tested. However, variation in the resistance patterns was observed with other classes of antimicrobial agents between the three lineages. The CC22 isolates (63/105; animals, $n=48$; humans, $n=15$) were resistant to one or more of the following classes; fluoroquinolones, lincosamines and/or tetracyclines and did not exhibit resistance to more than three classes of antimicrobial agents. Thirty-seven of the 63 CC22 isolates (animals, $n=27$; humans, $n=10$) exhibited the same AR profile as the current MRSA strain prevalent in Irish hospitals AR06, which is similar to the UK epidemic strain EMRSA-15 [8]. The remaining 26 isolates (animals, $n=21$; humans, $n=5$) were not assigned an AR type (NT) and required PFGE for classification [8].

The CC8 isolates (35/105; animals, $n=23$; humans, $n=12$) were resistant to aminoglycosides, tetracyclines and trimethoprim/sulfamethoxazole and were variably resistant to fluoroquinolones, lincosamines and rifampicin. The CC5 isolates (7/105; animals, $n=6$; humans, $n=1$) were resistant to aminoglycosides and macrolides. CC8 and CC5 AR patterns were designated 'Unfamiliar' as these patterns were unlike any of the AR patterns detected in recent years by NMRSARL [18].

The PFGE patterns obtained could be broadly divided into three types; A, B and C (Fig. 1). Pattern A was associated with CC22 isolates only, pattern B with CC8 isolates and pattern C with CC5 isolates.

Of the 63 CC22 isolates 47 exhibited a single PFGE pattern (pattern A) with the remaining 16 showing PFGE profiles differing by 4–6 bands which by application of the Tenover criteria suggests that they are 'possibly related' [19]. The Irish NMRSARL has an extensive database of AR- and PFGE-typing patterns of MRSA isolates recovered from human patients in Ireland in recent years [8]. PFGE patterns are assigned five-digit pulsed-field type (PFT) numbers which are abbreviated to two-digit PFT groups (PFG) following visual inspection of the gel images [8]. AR- and PFGE-typing results are combined to give AR-PFG types. The most frequently occurring pattern in isolates recovered from humans in Ireland is AR-PFG 06-01. The diversity seen in PFGE patterns in AR-PFG 06-01 isolates has increased from seven patterns in 1999 to 50 patterns at present [18]. Pattern A, as observed in the CC22 isolates in our study was indistinguishable from PFT 01018, the predominant pattern produced by Irish human MRSA isolates belonging to AR type AR06.

Nineteen of the 35 CC8 isolates exhibited a single PFGE pattern (pattern B) with the remainder (B1–B4) considered closely related based on minor band differences. Patterns A and B have been previously described in pet and in equine isolates in Ireland [20]. A single pattern (pattern C) was associated with the CC5 isolates.

Fifty-two of the MRSA isolates described in this study (animals, $n=34$; humans, $n=18$) were *spa*-typed. Thirteen of these isolates had been *spa*-typed in a previous study [16]. Nine *spa* types were detected in the isolates and subsequent BURP analysis assigned eight to one of three *spa*-derived clonal complexes with one singleton *spa* type, t1802 (Fig. 2). The *spa* types detected have an inferred association with multilocus sequence typing (MLST) clonal complexes; CC22, CC8 and CC5 [21]. The outlier t1802 was assigned to CC22 based on its PFGE pattern (pattern A). Four different *spa* types were detected in the CC22 isolates, the most frequent being t032 (14/20) which was detected in eight canine, five human and one equine isolate. Three *spa* types were observed in the CC8 isolates with t064 (13/27) and t451 (12/27) being the most frequent. The majority of human isolates within this group were *spa* type t064 (9/10) while type t451 accounted for 11/17 equine isolates. The three equine, one canine and one human CC5 isolates were all *spa* t002.

The three *spa* clonal complexes (*spa* CC) showed complete congruence with the three AR/PFGE groupings. Two of these lineages have been previously described in Irish isolates [20] but the third is a novel finding.

DISCUSSION

Phenotypic and molecular characterization of the MRSA isolates in this study has demonstrated the presence of three distinct MRSA genetic lineages in animals and veterinary personnel in Ireland. One lineage appears to be associated mainly with cats and dogs (CC22), the second with horses (CC8) and the third (CC5) affecting both horses and dogs. All three lineages have been identified in nasal swabs from

Table 1. *Summary of results relating to the 52 MRSA isolates characterized by AR, PFGE and* spa*-typing and the associated inferred multilocus sequence typed clonal complexes*

Clonal complex	*spa* types	PFGE types	AR types
CC22	t032 ($n=14$)	A ($n=10$)	AR06* [ApCp] ($n=1$), [ApCdCp] ($n=1$). 'NT'† [ApCdCpErLnTe] ($n=3$), [ApCdErLn] ($n=3$), [ApCdErLnSt] ($n=1$), [ApCdCpFdSt] ($n=1$)
		A1 ($n=4$)	AR06 [ApCpEr] ($n=1$), [ApCdCpEr] ($n=2$), 'NT' [ApCdCpErLn] ($n=1$).
($n=20$)			
	t022 ($n=3$)	A ($n=3$)	AR06 [ApCpEr] ($n=3$)
Horse (1 cs§)			
	T005 ($n=1$)	A1 ($n=1$)	AR06 [Ap]
Dogs (11 cs, 1 nc‖)			
	T749 ($n=1$)	A ($n=1$)	'NT' [ApCdCpErLn]
Humans (7 nc)			
	T1802 ($n=1$)	A7 ($n=1$)	'NT' [ApCpErFdLn]
CC8			Unfamiliar‡
		B ($n=1$)	Ap……ErGnKn…Nm…SuTbTeTp
		B1 ($n=3$)	
			Ap……ErGnKn…….StSuTbTe ($n=1$)
			Ap……ErGnKn……SuTbTe ($n=1$)
			Ap……ErGnKn…….Rf.SuTbTe ($n=1$)
		B2 ($n=3$)	Ap……ErGnKn…….RfSuTbTeTp ($n=3$)
($n=27$)	T064 ($n=13$)		
		B3 ($n=4$)	Ap…….GnKn……..StSuTbTe ($n=1$)
Horse (13 cs, 4 nc)			Ap…….ErGnKn…….RfSuTbTeTp ($n=2$)
			Ap…….ErGnKn……SuTbTeTp ($n=1$)
Humans (10 nc)			
		B5 ($n=1$)	Ap…….GnKn…….RfSuTbTeTp ($n=1$)
		B4 ($n=1$)	Ap…….ErGnKn……SuTbTeTp ($n=1$)
		B ($n=12$)	
			Ap……Kn……SuTbTeTp ($n=1$)
			Ap……..ErGnKnLnNm….SuTbTeTp ($n=1$)
			Ap…….ErGnKn….Nm…..SuTbTeTp ($n=2$)
	T451 ($n=12$)		Ap…EbErGnKn…Nm…….SuTbTeTp ($n=1$)
			Ap…EbErGnKnLnNm…….SuTbTeTp ($n=2$)
			Ap…EbErGnKnLnNmRf….SuTbTeTp ($n=3$)
			Ap…EbErGnKn…NmRf…..SuTbTeTp ($n=2$)
	t394 ($n=2$)	B5 ($n=2$)	Ap…….ErGnKn……..Rf….SuTbTeTp ($n=2$)
CC5	T002	C ($n=5$)	Ap…….ErGnKn……Tb ($n=5$)
($n=5$)			
Horses (2 cs, 1 nc)			
Canine (1 cs)			
Human (1 nc)			

Ap, ampicillin; Cd, cadmium nitrate; Cp, ciprofloxacin; Er, erythromycin; Fd, fusidic acid; Gn, gentamicin; Kn, kanamycin; Ln, lincomycin; Nm, neomycin; Rf, rifampicin; Su, sulphonamide; St, streptomycin; Tb, tobamycin; Te, tetracycline; Tp, trimethoprim.

* AR06, pattern observed in prevalent EMRSA strain in Ireland.

† NT, Isolates not assigned an AR type/designated 'No Type' as such isolates require PFGE typing for characterization [8].

‡ Unfamiliar: pattern unlike any patterns in the NMRSARL database [20].

§ cs, Clinical case.

‖ nc, Non-clinical/asymptomatic carrier.

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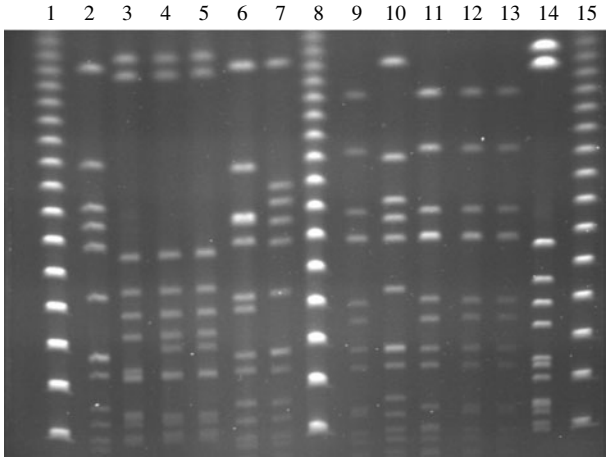

Fig. 1. PFGE analysis of MRSA isolates following macrorestriction of genomic DNA with *Sma*I showing PFGE patterns A, B and C. Lanes 1, 8, 15, DNA size marker; lanes 2, 10, pattern B2; lane 3, pattern A; lanes 4, 5, pattern A4; lane 6, pattern B3; lane 7, pattern B5; lanes 9, 11–13, pattern C; lane 14, NCTC 8325 *Staph. aureus*.

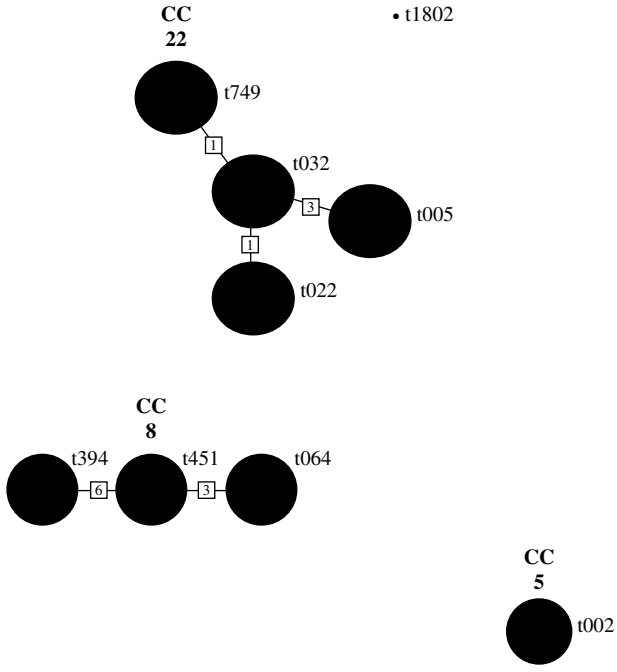

Fig. 2. BURP analysis of the identified *spa* types showing three *spa* clonal complexes and a singleton *spa* type, t1802.

veterinary personnel working with these cases. The isolates affecting pets were similar to the prevalent EMRSA strain (CC22) found in Irish healthcare institutions. It is likely that these CC22 isolates are the result of an overspill from the human hospital environment. In contrast, CC5 and CC8 isolates, have only recently been reported in humans in Ireland [18].

Sixty percent (63/105) of the isolates described in this study belonged to CC22. The animals in this group were mainly cats and dogs, with only one isolate from a horse. These animal isolates included one from a healthy dog. The human isolates ($n=15$) in this group were isolated from staff who worked in close proximity to these animals. Only one person provided information regarding a healthcare-acquired (HCA) risk factor, which may have contributed to its acquisition.

Thirty-seven CC22 isolates were indistinguishable from the predominant MRSA strain found in human patients in Ireland. Designated AR-PFG type 06-01 by NMRSARL, this strain currently accounts for 84% (343/407) of isolates investigated in the human population in 2008 in Ireland and resembles the predominant UK strain EMRSA-15 [8]. The remaining CC22 isolates ($n=26$) were deemed to be closely related variants. To the best of our knowledge, no other report has described streptomycin resistance in MRSA strains isolated from pets. This antibiotic is not licensed for use in the treatment of infections in dogs or cats in Ireland. The most frequent *spa* type in the CC22 isolates was t032, also previously described in pet animals [16] and one of the most common *spa* types in Irish human MRSA strains investigated in recent years [18].

The CC8 isolates included 23 equine and 12 human MRSA isolates. Seven of the 12 humans were veterinary staff who worked in close proximity with MRSA-positive horses. The equine isolates included four isolates from colonized healthy animals. The typing results revealed a highly resistant AR pattern, with resistance to as many as 13 antimicrobial agents. The PFGE patterns differed from those of AR-PFG 06-01, by more than six bands and were therefore considered unrelated. Representative isolates of this group were found to be indistinguishable from Canadian equine isolates using PFGE (J. S. Weese, personal communication, 2004).

The strains of MRSA which colonize and infect horses are frequently different from the concurrent circulating human strains [22]. Isolates similar to the Canadian epidemic MRSA 5 strain (CMRSA-5) were first described in horses in Canada in 2000. This strain is relatively uncommon in North America [23] but appears to be the predominant strain in horses in Canada and has been shown to be the most prevalent global isolate in horses [5]. The wide dissemination of this strain in the Irish equine population supports the notion of host adaptation of this clone (J. S. Weese, personal communication, 2004).

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Until recently, *spa* type t064 had not been observed in the human isolates investigated by NMRSARL. However, in 2007 NMRSARL investigated a collection of 86 MRSA isolates that exhibited unusual AR or PFGE patterns and *spa* type t064 was one of three common *spa* types within this group. Two thirds of the *spa* type t064 isolates were recovered from isolates from horses or patients with a history of contact with horses [18]. To date *spa* t451 has not been observed in MRSA isolates within the Irish human population. NMRSARL reported only one isolate of *spa* type t394, originating from a veterinary source, in its database [18].

The CC5 isolates had distinct AR, PFGE and *spa*-typing results, not previously encountered in animal MRSA isolates in Ireland. These isolates may be related to the Canadian epidemic strain 2 (CMRSA-2, *spa* t002 ST5) which has been reported as the predominant strain in the companion animal population in Canada [6]. CMRSA-2 is related to the USA 100 clone otherwise referred to as the New York or Japanese clone and is the most common cause of community-associated MRSA infection in Canada [6]. MRSA *spa* type t002 has been associated with clinical infections in dogs, cats and horses [6] and more recently with an outbreak involving marine animals in a marine park [24]. This *spa* type has also been reported in human MRSA isolates in Central Europe, Japan and southern Korea [25]. The NMRSARL in Ireland has nine *spa* t002 isolates in its collection, two of which are from patients with equine associations [18].

It has been suggested that zoonotic colonization of individuals working in close contact with animals, could provide the initial point of contact for entry of novel MRSA strains into the human population [26]. Recent reports from Germany, Malta and Tenerife suggest that international travel by people may contribute to the dissemination of strains from one country to another [27, 28]. The international travel of horses, a feature of the equine industry, may be an additional means of spread of MRSA strains. Ireland has the most horse-population density in Europe, with about 45 000 thoroughbred and 110 000 sports horses resident in the country [29]. Horses are transported on a temporary or permanent basis between countries and continents for breeding, racing or show-jumping purposes. Two thousand and eighty-six horses from USA and 1729 from France and Germany were permanently imported into Ireland over the past 4 years. The movement of horses differs from other farm species which are frequently transported for slaughter purposes only. A recent report from the UK identified two horses with the MRSA strain ST398. One horse was a recent import from Spain via France while the second animal had no history of being abroad [30]. MRSA ST398 is mainly associated with the pig industry but has also been reported in veal calves and in poultry [31].

In contrast, it has been suggested recently that lineages of *S. aureus* are largely endemic within individual geographical areas and that spread to other countries or continents occurs only on rare occasions [25]. Research into a population of 135 MRSA MLST (CC5) strains, including *spa* t002 isolates from 22 countries on four continents, indicated that MRSA has emerged independently on numerous occasions in several countries and that geographical dispersal is limited. This conclusion of convergent evolution is contrary to the widely held opinion that emergence of MRSA epidemic clones has only occurred on a small number of occasions and that these clones have been spread globally [32]. The present finding of significant similarity between Irish and Canadian equine isolates tends to support the latter view; however, further genetic studies are required, preferably whole genome sequence comparison, to substantiate this. It is also possible that different clonal complexes have evolved along different lines.

Most veterinary practitioners associate MRSA in animals with 'hospital-associated' or HA-MRSA strains and are unaware of other potential sources. In addition asymptomatic carriage of MRSA in healthy animals and in people has been described as a strong predictor of infection for both animals and humans [33, 34]. Farm workers, pet owners and veterinary personnel have all been associated with the transmission of MRSA between animals and humans [7, 35]. In particular, the emergence of MRSA CC398 in pigs and its subsequent spread to humans has demonstrated the potential for animals to act as reservoirs in the transmission and spread of particular MRSA strains [7].

The present study has described the emergence of three different clonal lineages of MRSA present in animals and veterinary personnel in Ireland. Two of these, CC5 and CC8, appear to be non-endemic clones and their introduction may possibly be linked to the international equine trade. Additional molecular comparison of these CC5 and CC8 isolates to international MRSA animal and human strains would be necessary in order to determine their origin. We highlight the importance of considering animal

movement between countries, when investigating the spread of new MRSA strains in animals and humans.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Baptiste KE, *et al.*** Methicillin-resistant staphylococci in companion animals. *Emerging Infectious Diseases* 2005; **11**: 1942–1944.
2. **Goni P, *et al.*** Antibiotic resistance and epidemiological typing of *Staphylococcus aureus* strains from ovine and rabbit mastitis. *International Journal of Antimicrobial Agents* 2004; **23**: 268–272.
3. **Walther B, *et al.*** Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Veterinary Microbiology* 2008; **127**: 171–178.
4. **Rich M, Roberts L, Kearns A.** Staphylococci in animals: prevalence, identification and antimicrobial susceptibility, with an emphasis on methicillin-resistant *Staphylococcus aureus*. *British Journal of Biomedical Science* 2005; **62**: 98–105.
5. **Morgan M.** Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? *Journal of Antimicrobial Chemotherapy* 2008; **62**: 1181–1187.
6. **Weese JS, *et al.*** Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Veterinary Microbiology* 2006; **115**: 148–155.
7. **van Loo I, *et al.*** Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerging Infectious Diseases* 2007; **13**: 1834–1839.
8. **Rossney A, *et al.*** Epidemiological typing of MRSA isolates from blood cultures taken in Irish hospitals participating in the European Antimicrobial Resistance Surveillance System 1999–(2003). *European Journal of Clinical Microbiology & Infectious Diseases* 2006; **25**: 79–89.
9. **Murchan S, *et al.*** Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *Journal of Clinical Microbiology* 2003; **41**: 1574–1585.
10. **Harmsen D, *et al.*** Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *Journal of Clinical Microbiology* 2003; **41**: 5442–5448.
11. **Abbott Y, *et al.*** Isolation rates of meticillin-resistant *Staphylococcus aureus* (MRSA) in pets and horses in Ireland. *Veterinary Record* (in press).
12. **Raus J, Love DN.** Characterization of coagulase-positive *Staphylococcus intermedius* and *Staphylococcus aureus* isolated from veterinary clinical specimens. *Journal of Clinical Microbiology* 1983; **18**: 789–792.
13. **Clinical and Laboratory Standards Institute (CLSI).** Performance standards for antimicrobial disk diffusion susceptibility tests; Approved Standard – Ninth Edition. Fifteenth Informational Supplement M100-S15, Clinical and Laboratory Standards Institute 2005, Wayne, PA, 19087-1898.
14. **Leonard RB, Carroll KC.** Rapid lysis of gram-positive cocci for pulsed-field gel electrophoresis using achromopeptidase. *Diagnostic Molecular Pathology* 1997; **6**: 288–291.
15. **Duck WM, *et al.*** Optimization of computer software settings improves accuracy of pulsed-field gel electrophoresis macrorestriction fragment pattern analysis. *Journal of Clinical Microbiology* 2003; **41**: 3035–3042.
16. **Moodley A, *et al.*** spa typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. *Journal of Antimicrobial Chemotherapy* 2006; **58**: 1118–1123.
17. **Mellmann A, *et al.*** Based upon repeat pattern (BURP): an algorithm to characterize the long-term evolution of *Staphylococcus aureus* populations based on spa polymorphisms. *BMC Microbiology* 2007; **7**: 98.
18. **Anon.** Annual report (2008). Dublin, Ireland: Irish National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory (NMRSARL), St James's Hospital, Dublin, Ireland, 2008.
19. **Tenover FC, *et al.*** Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* 1995; **33**: 2233–2239.
20. **O'Mahony R, *et al.*** Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology* 2005; **109**: 285–296.
21. **Strommenger B, *et al.*** *spa* typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. *Journal of Clinical Microbiology* 2008; **46**: 574–581.
22. **Cuny C, *et al.*** Emergence of MRSA infections in horses in a veterinary hospital: strain characterisation and comparison with MRSA from humans. *Eurosurveillance* 2006; **11**: 44–47.
23. **Weese JS, *et al.*** Community-associated methicillin-resistant Staphylococcus aureus in horses and humans who work with horses. *Journal of the American Veterinary Medical Association* 2005; **226**: 580–583.
24. **Faires M, Weese JS.** An investigation of a methicillin-resistant *Staphylococcus aureus* outbreak in marine animals. In: *Proceedings of the 19th European Congress of Clinical Microbiology and Infectious Diseases*. Helsinki, Finland, 2009, p. 1381.

25. **Nubel U, *et al.*** Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. In: *Proceedings of the National Academy of Sciences USA* 2008; 105: 14130–14135.
26. **Stein RA.** Methicillin-resistant *Staphylococcus aureus* – the new zoonosis. *International Journal of Infectious Diseases* 2009; **13**: 299–301.
27. **Gould SWJ, *et al.*** UK epidemic strains of methicillin-resistant *Staphylococcus aureus* in clinical samples from Malta. *Journal of Medical Microbiology* 2008; **57**: 1394–1398.
28. **Montesinos I, *et al.*** Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* associated with the emergence of EMRSA-16 at a university hospital. *Journal of Hospital Infection* 2006; **64**: 257–263.
29. **Collins J, *et al.*** The structure and regulation of the Irish equine industries; links to considerations of equine welfare. *Irish Veterinary Journal* 2008; **61**: 746–756.
30. **Loeffler A, *et al.*** First isolation of MRSA ST398 from UK animals: a new challenge for infection control teams? *Journal of Hospital Infection* 2009; **72**: 269–271.
31. **van de Giessen AW, *et al.*** Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. *Preventive Veterinary Medicine* 2009; **91**: 270–273.
32. **Deurenberg RH, *et al.*** The molecular evolution of methicillin-resistant. *Staphylococcus aureus Clinical Microbiology and Infection* 2007; **13**: 222–235.
33. **Weese JS, *et al.*** Methicillin-resistant *Staphylococcus Aureus* in horses at a veterinary teaching hospital: Frequency, characterization, and association with clinical disease. *Journal of Veterinary Internal Medicine* 2006; **20**: 182–186.
34. **Robicsek A, *et al.*** Prediction of methicillin-resistant *Staphylococcus aureus* involvement in disease sites by concomitant nasal sampling. *Journal of Clinical Microbiology* 2008; **46**: 588–592.
35. **Manian FA.** Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clinical Infectious Diseases* 2003; **36**: 26–28.