Short Communications

Prevalence of meticillin-resistant staphylococci among dogs and cats at a veterinary teaching hospital in Portugal

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THE emergence of meticillin-resistant *Staphylococcus aureus* (MRSA) and meticillin-resitant *Staphylococcus pseudintermedius* (MRSP) in pet animals is a public health concern due to the high zoonotic potential of these multidrug-resistant bacteria (Perreten and others 2010, Ruscher and others 2010, Weese and van Duijkeren 2010). Major human epidemic clones of MRSA, mainly sequence type (ST) 22 (EMRSA-15), have been isolated from dogs and cats in various countries (Weese and van Duijkeren 2010). Although rare, human infections with MRSP of canine origin have recently been reported (Van Hoovels and others 2006, Chuang and others 2010) and high carriage rates have been shown among small animal practitioners in the USA (Morris and others 2010).

As data on MRSA and MRSP carriage rates in dogs and cats are scarce, particularly in Europe, this study investigated the prevalence of MRSA and MRSP carriers among animals presenting at the teaching hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal, thus representing a sample from a tertiary referral centre population.

From April 2008 to May 2010, nasal swabs were obtained from 141 cats and 146 dogs within two hours after admission to the hospital. The swabs were enriched in 3 ml broth containing 10 g/l mannitol, 65 g/l sodium chloride, 2.5 g/l yeast extract and 10 g/l tryptone. After overnight incubation, 10 µl bacterial suspension was plated on to a selective medium (ChromID MRSA; bioMérieux) and suspected MRSA/MRSP colonies were confirmed by PCR detection of *mecA* (Technical University of Denmark [DTU] 2008). Meticillin-resistant staphylococci were isolated from 10 dogs (7.1 per cent) and two cats (1.4 per cent).

Based on the *nuc* multiplex PCR (Sasaki and others 2010), nine isolates were identified as MRSP and the remaining three isolates were determined to be MRSA. MRSP was detected only in dogs (6.2 per cent, 95 per cent confidence interval [CI] 2.9 to 11.4 per cent), resulting in a statistically significant difference between dogs and cats (Fisher's exact test, P=0.004). The odds of a dog being MRSP-positive were 12.7 times the odds for a cat. This finding was not unexpected, as it reflects the more frequent occurrence of *S pseudintermedius* in dogs compared with cats (Moodley and others 2009, Ruscher and others 2010). Although carriage of MRSP appears to be rare in cats, MRSP infections and carriage in healthy cats have been reported in Europe and Canada (Kadlec and others 2010, Pomba and others 2010).

All isolates were characterised genetically by pulsed-field gel electrophoresis (PFGE) (Perreten and others 2010), *spa* typing (Moodley and others 2009, Perreten and others 2010), SCC*mec* typing (Kondo and others 2007) and detection of *lukF/lukS* (Lina and others 1999, Ruscher and others 2010). Two distinct genetic lineages were identified among the nine MRSP isolates (Table 1): PFGE type A-*spa* type t06-SCC*mec* V-Luk-I-negative-*Staphylococcus intermedius* exfoliative toxin (SIET)-positive (one isolate) and PFGE type B-*spa* type t02-SCC*mec* III-Luk-I-positive-SIET-positive (eight isolates). PFGE type A displayed the typical genetic characteristics of the American epidemic clone (Black and others 2009, Perreten and others 2010), whereas PFGE type B corresponded to the European epidemic clone (Perreten and others 2010, Ruscher and others 2010). In 2009, the first MRSP infection was identified in a lower urinary tract infection in a cat in Portugal; this isolate was associated with the American epidemic MRSP clone (Pomba and others 2010). Apart from Portugal, this clone had been exclusively reported in the USA and Canada (Black and others 2009, Moodley and others 2009, Perreten and others 2010). The presence of Luk-I and SIET in MRSP ST71 has been previously described (Ruscher and others 2010). Luk-I shows a strong toxicity on various polymorphonuclear cells, including human leucocytes, and SIET has an exfoliative effect on dogs, with development of clinical signs similar to the signs of canine pyoderma (Ruscher and others 2010). Since phagocytosis by polymorphonuclear cells is an important defence mechanism of the host, Luk-I may play a primary role in MRSP ST71 evasion of the host defence mechanisms.

MRSA was found in two cats (1.4 per cent, 95 per cent CI 0.2 to 5.0 per cent) and one dog (0.7 per cent, 95 per cent CI 0.02 to 3.8 per cent). No statistically significant difference was observed between the two host species in relation to MRSA carriage. The three MRSA isolates belonged to *spa* type t032, harboured SCC*mec* type IV, were PVL-negative and displayed a unique pattern on PFGE corresponding to EMRSA-15 (ST22) (Table 1). This MRSA lineage has previously been associated with small animals in the UK and Ireland (Loeffler and others 2005, Moodley and others 2006). EMRSA-15 is a well-known MRSA clone causing infections in people, and it has therefore been hypothesised that the presence of this clone in small animals is likely to have been acquired from human beings (Weese and van Duijkeren 2010). The prevalence of MRSA among people in Portugal remains one of the highest in Europe (European Centre for Disease Prevention and Control [ECDC] 2007), and EMRSA-15 is the most common clone in the country (Aires-de-Sousa and others 2008).

Antimicrobial susceptibility testing was performed by the disc diffusion method (Oxoid) according to Clinical and Laboratory Standards Institute (CLSI) guidelines M31-A3 (CLSI 2008, 2009). MRSP isolates were resistant to a larger number of antibiotics than the MRSA isolates, which were consistently resistant to fluoroquinolones in addition to β-lactams (Table 1). This finding confirms that MRSP may represent a major therapeutic challenge for veterinarians due to a limited choice of antibiotics. The antibiotics to which MRSP remains susceptible are not licensed for use in small animals (eg, amikacin, chloramphenicol and nitrofurantoin), are used for human decolonisation (fusidic acid and mupirocin) or are last-resort antimicrobials for the treatment of MRSA infections in human medicine (linezolid, quinopristin/dalfopristin, rifampicin and vancomycin) (Food and

Veterinary Record (2011) 169, 72a doi: 10.1136/vr.c6948

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Provenance: not commissioned; externally peer reviewed

Published Online First April 18, 2011

TABLE 1: Molecular characteristics and antimicrobial resistance profiles of meticillin-resistant *Staphylococcus pseudintermedius* (FMV 1 to 9) and meticillin-resistant *Staphylococcus aureus* (FMV 10 to 12) isolates from cats and dogs presented at the teaching hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal

Strain	Origin	Antimicrobial-associated co-resistance patterns	SCC*mec* type	*spa* type	Toxins identified	CC	PFGE subtype
FMV 1	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, E^R, W^R	V	t06	SIET	68	B
FMV 2	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A1
FMV 3	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A2
FMV 4	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A1
FMV 5	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A1
FMV 6	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A3
FMV 7	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A1
FMV 8	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A1
FMV 9	Dog	Cn^R, K^R, Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R, Da^R, E^R, Te^R, W^R	III	t02	SIET, Luk-I	71	A1
FMV 10	Cat	Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R	IV	t032	None	22	C
FMV 11	Cat	Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R	IV	t032	None	22	C
FMV 12	Dog	Cip^R, Enr^R, Lev^R, Mar^R, Mxf^R, Nor^R, Ofx^R	IV	t032	None	22	C

Cip Ciprofloxacin, Cn Gentamicin, Da Clindamycin, E Erythromycin, Enr Enrofloxacin, K Kanamycin, Lev Levofloxacin, Mar Marbofloxacin, Mxf Moxifloxacin, Nor Norfloxacin, Oxf Ofloxacin, PFGE Pulsed-field gel electrophoresis, SCC Staphylococcal chromosome cassette, SIET *Staphylococcus intermedius* exfoliative toxin, Te Tetracycline, W Trimethoprim

Agriculture Organization/World Organisation for Animal Health/World Health Organization [FAO/OIE/WHO] 2008). The situation is further complicated by the fact that some of these antibiotics may cause adverse reactions and have poor pharmacokinetic properties in small animals (Perreten and others 2010). This scenario represents a serious ethical dilemma, even when applying the cascade principle (FAO/OIE/WHO 2007). Since there are no guidelines for treatment of MRSP infections, it is up to individual veterinarians to decide which antimicrobial to use.

In conclusion, the MRSP carriage rate (6.2 per cent) observed in dogs in this study was relatively high considering that MRSP was first described in Europe in 2006 and in Portugal in 2009. These data suggest that these bacteria are spreading through the canine population throughout Europe, including Portugal. In contrast to other European countries, it appears that both the European (ST71) and the American (ST68) epidemic clones are circulating in the dog population in Portugal. The prevalence of MRSA carriage is lower than that of MRSP and the genetic background of MRSA isolates from pets corresponds to ST22 (EMRSA-15), which is the predominant lineage among human clinical isolates in Portugal and has been previously reported in pets in other countries.

Acknowledgements

This study was supported by the Interdisciplinary Centre of Research in Animal Health from Fundação para a Ciência e a Tecnologia, Portugal, and by the Faculty of Life Sciences, University of Copenhagen, Denmark. The authors thank colleagues at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, and Filipa Baptista for helping with the statistical analysis.

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Veterinary Record 2011 169: 72 originally published online April 18, 2011
doi: 10.1136/vr.c6948