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Short communication

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations

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Abstract

Clinical specimens of small animals (n=869) were screened for the occurrence of methicillin-sensitive and methicillin-resistant *S. aureus* (MSSA; MRSA) during routine microbiological examinations, and results were confirmed by a multiplex PCR strategy. The genetic relatedness of all *mec*A-positive *S. aureus* isolates was further investigated by pulsed-field gel electrophoresis (PFGE), multi locus sequence typing (MLST), PCR for Panton- Valentine leukocidine genes (PVL) and staphylococcal cassette chromosome *mec*- typing (SCC*mec*).

A total of 61 *S.aureus* isolates were found during a 20-month period of investigation, 27 (44.3%) of them harbouring the *mec*A gene for methicillin-resistance. The majority of MRSA were isolated in specimens from dogs (n=18) and cats (n=4). One guinea pig and one rabbit were found to be positive for an MRSA infected site. Similarly, three exotic animals, a turtle, a bat and a parrot, were found to be infected with MRSA. PFGE and MLST analysis revealed a certain genotype ("A", and "A-1") dominating the isolate collection (23 of 27)**.** Furthermore, one isolate showed homologous PFGE pattern to the German epidemic strain Barnim ("BE") and another one ("BE-1") was considered to be closely related. A third genotype ("B") was detected in two cases. Two different sequence types (ST) were identified among the 27 MRSA isolates. PFGE type "A" and both strains related to the Barnim epidemic strain were assigned to ST22, whereas ST239 was associated to PFGE profile "B".

The present data show that certain MRSA genotypes are capable of infecting a wide spectrum of small and exotic animals, especially in clinical facilities.

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Introduction

Among pathogens causing infectious diseases, *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), are an important cause of human infectious diseases. In Germany, the MRSA situation in hospitals and other community health settings varies regionally. A validated surveillance study (SARI) reported about cumulative MRSA percentages ranging from 0% to 64,4% in German intensive care units (Meyer et al., 2006).

Since the mid 1970`s, cases of infections due to MRSA were reported in different animal species (Devriese and Hommez, 1975; Hartmann et al., 1997; Pak et al., 1999; Shimizu et al., 1997). Meanwhile, MRSA infections in domestic animals have been reported among horses, pigs, cattle, sheep, cats, dogs and rabbits. In the last years however, there has been an increase in reports on MRSA infections in small animals and horses (Middleton et al., 2005; Rich and Roberts, 2006; Seguin et al., 1999; Walther et al., 2006). Moreover, occurrence of MRSA has been reported as a problem in veterinary (teaching) facilities (O'Mahony et al., 2005; Strommenger et al., 2006; Weese et al., 2006).

In April 2003, a dog suffering from delayed wound healing after surgery due to MRSA attracted our attention. The animal was a patient of the small animal hospital at the Free University Berlin. We decided to perform a screening for MRSA in addition to routine diagnostic procedures on all microbiological specimens originating from small animal patients of that facility. A second aim was to analyze the genetic relatedness of collected MRSA isolates using common typing techniques, including pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), Staphylococcal cassette chromosome *mec*- (SCC*mec*) typing and PCR detection of the genes encoding for the Panton-Valentine leukocidine (PVL) factor.

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Materials and methods

Setting

All microbiological specimens reported on here were delivered from the veterinary hospital for small animals at the Free University of Berlin. This clinical facility has a case load of about 30,000 small and exotic animals per year. Animals with all kinds of diseases are admitted to this veterinary hospital, most of them being out patients.

Bacterial isolation and identification

The institute for microbiology and epizootics (IMT), part of the Free University Berlin, provides microbiological diagnostic services for the different veterinary clinical teaching departments of the university and for private veterinary hospitals or smaller settings.

A total of 869 clinical specimens, which were routinely sent in for diagnostic purposes between May 2003 and December 2004, were investigated in this study. All clinical specimens originated from small animal patients of the veterinary teaching hospital. The whole spectrum of animal species and diagnostic material (origin of specimens) investigated per species is given in table 1.

All specimens were routinely inoculated onto the following media: Standard nutrient agar I (Roth GmbH, Karlsruhe, Germany) with 5% defibrinated sheep blood, Chrom agar orientation (Mast Diagnostica, Reinfeld, Germany) and Gassner agar (Sifin, Berlin, Germany). All plates were investigated twice, first after 18 h and a second time after 36h of incubation at 37°C. Staphylococcal isolates were identified by morphology on agar plates, Gram stain appearance, catalase test, the tube coagulase reaction and their ability to ferment mannitol anaerobic (Mossel, 1962; Songer and Post 2005).

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All coagulase-positive *Staphylococcus spp*. isolates were routinely investigated by PCR, regardless of other phenotypic test results. Genotypic confirmation of *S. aureus* was implemented by amplification of a species-specific sequence of the thermostable nuclease (*nuc*) and methicillin-resistance was diagnosed by detecting the *mec*A-gene responsible for broad spectrum ß-lactam-resistance in staphylococci (Merlino et al., 2002).

Typing of MRSA strains

PFGE

PFGE analysis was used to provide a DNA fingerprint profile from all MRSA isolates which were collected during this study (n=27). Briefly, macrorestriction of genomic DNA was performed by using endonuclease *Sma*I. Restriction fragments were separated in a 1% agarose gel (pulsed- field certified Agarose, Bio-Rad Laboratories, München, Germany) and resolved on a CHEF DR II apparatus (Bio-Rad Laboratories) according to Mulvey et al., 2001.

The relatedness of PFGE types was determined by visual comparison and by employing the software GelCompar II (Applied Math, Kortrijk, Belgium) for the percent similarity calculation (Dice coefficient, tolerance 1%, optimization 0,5%) of all MRSA isolates and named according to previously described principles (Tenover et al., 1995).

MLST

MLST typing of seven housekeeping genes was carried out for all different PFGE types (n= five types: A, A1, B, BE, BE-1) as described elsewhere (Enright et al., 2002).

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SCC*mec*

SCC*mec*- typing was carried out for all MRSA isolates (n= 27) by using a multiplex PCR strategy according to Oliveira and de Lencastre, 2002.

Detection of PVL genes

PCR detection of Panton-Valentine leukocidin (PVL) genes (*luk*S-PV-*luk*F-PV) was carried out as previously described by Lina et al., 1999.

Results

During the 20 month investigation period from May 2003 to December 2004 we screened 869 clinical specimens, which had been delivered to the IMT microbiological laboratory for routine diagnostic purposes. The proportion of specimens for each animal species varied extensively. The absolute majority of specimens were from dogs (67.8%), followed by specimens from cats (17%), birds (6.8%), rabbits (3.3%), guinea pigs (2.5%) and other small and exotic animals. All data including source of animal specimens and body site of origin of all 869 specimens are provided in table 1.

A total of 61 *S. aureus* isolates, i.e. 34 (55.7%) MSSA and 27 (44.3%) MRSA, were found in specimens obtained from different animal species (Tab.2). The general *S. aureus* (MSSA/MRSA) frequency in clinical specimens from dogs was 5.4% and from cats 7.4%. Frequency rates of *S. aureus* obtained from specimens of other animal species were: birds 11.9%, rabbits 6.9%, and guinea pigs 18.1%.

We did not calculate frequency rates for further animal species mentioned in table 1 (i.e. chinchilla, lizard, squirrel, bat, turtle, rat and snake) due to the limited number of specimens (<20/ animal species). All MRSA/MSSA isolates from the present study, sorted by animal species and specimen origin, are shown in table 2. The majority of

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MRSA isolates were detected in specimens from dogs (18), followed by those from cats (4), but also in one specimen each from a rabbit and a guinea pig. Surprisingly, MRSA was also detected in three specimens from exotic animals, i.e. a parrot suffering from osteomyelitis, a bat with wound infection as well as in a turtle with skin disease. All data concerning isolation time, animal host and site of specimen origin is shown in table 3.

Most of the MRSA isolates were obtained from wound infections (21 of 27; 77.8%). The infection rate due to MRSA in specimens from wound infections of dogs was 7.5% (15 of 201 specimens), in cats 10% (3 of 30 specimens) and in birds 2.3% (1 of 43). Other animals were not included in the calculation of wound infection rate due to the small number of wound infections (<20).

The average number of clinical MRSA infections in small animals was 1.35 per month during this study. The isolation rate was not constant during the investigated period. A sudden rise occurred twice, first in May 2004 and again in November 2004 (see Tab.3). In each of the two months mentioned, 7 new MRSA cases were registered.

Five different PFGE types were identified among the 27 MRSA isolates. Predominant was a type denominated as type "A", which was detected in 22 of 27 isolates (tab. 3) and once in an isolate assigned to be closely related ("A-1") (fig. 1). Also, with the exception of a single isolate, only PFGE type "A" was found in the both above mentioned increases of the MRSA isolation rate. MLST typing for isolates which showed PFGE type "A" or "A-1" assigned this clone to sequence type (ST) 22, the central genotype of clonal complex (CC) 22 (Enright et al., 2002). Another PFGE type of an isolate from a cat was indistinguishable from the PFGE pattern of the human epidemic strain Barnim (PFGE type BE in Table 3). Another isolate was found to be closely related (Barnim-1) (fig.1). Two further isolates displayed a third pattern, which

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was named "B" (fig.1). As a result of MLST analysis, this PFGE type was assigned to ST239, a genotype linked to CC8 (Enright et al., 2002).

Visual comparison of all representative PFGE types in this study with six further German human epidemic strains showed no similarities (fig.1).

SCC*mec*- typing revealed that PFGE type "A", "A-1", "BE" and "BE-1" was associated to SCC*mec* IV, whereas the SCC*mec* multiplex PCR for the PFGE profile “B” presented no definite results. The PCR showed a positive amplification signal for the loci C and H (related to SCC*mec* type IIIa). However, a positive signal for locus A (related to SCC*mec* type I) was also detected (Oliveira and de Lencastre, 2002). None of the MRSA isolates in the present study harbored the PVL genes.

Discussion

This study reports the occurrence of MRSA in clinical specimens derived from small animal patients of the Free University hospital in Berlin. A total of 61 *S. aureus* isolates were identified by PCR among the 869 specimens during a 20 month investigation period, 44.3% of these isolates additionally harboured the *mec*A gene for methicillin-resistance.

The incidence of MRSA infections in this study was 1.85 per 1.000 admissions. Weese et al. 2006 reported a similar rate of 1.8 per 1.000 admissions for nosocomial MRSA infections from horse patients of the Ontario Veterinary Teaching Hospital, Canada (Weese et al., 2006).

Remarkably, we did not only find MRSA from infected sites of small animals like dogs, cats, guinea pigs and rabbits, as has been reported by others (Baptiste et al., 2005; Boag et al., 2004; Strommenger et al., 2006; Weese, 2005), but also in more exotic animals like turtle, parrot and bat. A case of MRSA infection in a parrot has

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been reported before (Rankin et al., 2005), but to the best of our knowledge, infections due to MRSA in bats or turtles were unknown up to now.

We found a frequency of *S. aureus* in clinical specimens from dogs of 5.4% and of 7.4% for cats. These findings support the estimation, that the *S. aureus* frequency rate in clinical specimens from dogs and cats is typically below 10% (Leonard and Markey, 2007).

Without regard to animal species, MRSA infections were absolutely predominant in wound infections (77.7%) in this study. O`Mahony and coworkers (O'Mahony et al., 2005) reported a similar rate (77%) for non-equine animals.

The MRSA isolates collected in this study underwent different common typing procedures. PFGE analysis revealed, that 22 (+1 subtype: "A-1") of all 27 MRSA isolates could be assigned to the same PFGE type ("A"), including those from the three exotic animals mentioned. Thus, type "A", assigned to ST22, represents 85.2% of all MRSA isolates collected during the present study. Other studies have reported the frequent occurrence of MRSA isolates with a ST22 background in Northern Germany (Witte et al., 2001) human hospitals as well as in other university veterinary settings (Strommenger et al., 2006) for small animals. Furthermore, we have shown evidence for the occurrence of the human epidemic strain Barnim ("BE") as well as for a closely-related subtype designed as Barnim-1 ("BE-1") in animals (both: ST22). The first one was isolated from an auditory channel swab specimen from a cat, and the second one was detected in a wound specimen from a dog (Tab.2; fig.1). These findings support the theory on potential transferability of certain MRSA genotypes between animals and humans (Strommenger et al., 2006).

The two isolates which showed PFGE type "B" were assigned to ST239, which has been found in MRSA isolates from dogs in an Australian study (Malik et al., 2006).

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ST239 is also a well known genotype linked to MRSA isolates from human hospitals (Cirlan et al.).

SCC*mec*- typing showed a positive result for the smallest of these mobile genetic elements, SCC*mec* IV being associated to type "A" and A"-1". This finding was similar to that from Strommenger and coworkers (Strommenger et al., 2006), who found SCC*mec* IV in isolates of small animal origin with an ST22 background.

Multiplex PCR results of PFGE type "B" demonstrated no definite typing result with regard to a certain SCC*mec* type and we hypothesised a recombination event or mosaic structure for this genotype similar to recently published variants of SCC*mec* types (Hanssen and Sollid, 2007; Heusser et al., 2007).

It has recently been shown that *S. aureus* strains with invasive potential to humans and pets have arisen from the same genetic background (van Leeuwen et al., 2005). Furthermore, it is well known that the SCC*mec* complex, which harbours the resistance gene *mec*A, has a potential for being transferred horizontally (Enright et al., 2002).

As a conclusion of the present study it can be assumed that a single MRSA genotype, apparently well adapted to the local environmental conditions, appeared as a nosocomial pathogen in a small animal clinical facility, regardless of the animal species. Hospital aquired infections due to a single genotype have been reported earlier from veterinary settings (Leonard et al., 2006; Strommenger et al., 2006) and transmissions between man and animal seem to be likely (Baptiste et al., 2005; Manian, 2003; van Duijkeren et al., 2004), but environmental factors (Weese et al., 2004), also play a role in veterinary facilities.

Veterinarians should be aware of the potential nosocomial character of MRSA including small and exotic animal species. Therefore, we suggest general screening of MRSA in veterinary settings, particularly by introducing wound infection

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surveillance. The present data show that certain MRSA genotypes are able to infect a wide spectrum of small and exotic animals, especially in clinical facilities.

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Table 1: Distribution and source of animal specimens from routine diagnostic specimens (May 2003 to December 2004) by body site of origin.

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Table 2: Distribution of MRSA and MSSA in 61 *S. aureus*-positive specimens from infected sites of small and exotic animals

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Fig. 1. Dendrogramm (% similiarity) showing DNA restriction pattern after digestion with *Sma*I for all representative PFGE profiles (A, A-1, B, Barnim epidemic strain, Barnim-1) of this study in direct comparison to human epidemic strains with GelComparII using UPGMA; Dice coefficient, 1% tolerance and 0,5% optimisation.

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Table 3: Information on date of specimen arrival, animal species, kind of infected site and PFGE type from all 27 MRSA isolates reported on in this study

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Table 1

Animal species	n	specimen origin: Wounds (%)	Systemic infection [a] (%)	Respiratory system (%)	Skin and mucosa (%)	Urogenital tract (%)	Auditory channel (%)	Gastrointes-tinal tract (%)	Eye (%)
All species	869	309 (35.6)	93 (10.7)	48 (5.5)	83 (9.6)	204 (23.5)	97 (11.2)	28 (3.2)	7 (0.8)
Dog	589	201 (34.1)	63 (10.7)	32 (5.4)	56 (9.5)	156 (26.5)	56 (9.5)	22 (3.7)	3 (0.5)
Cat	148	30 (20.3)	17 (11.5)	13 (8.8)	12 (8.1)	46 (31.1)	26 (17.6)	2 (1.4)	2 (1.4)
Bird	59	43 (72.9)	8 (13.6)	2 (3.4)	2 (3.4)		4 (6.8)		
Rabbit	29	18 (62.1)	1 (3.4)	1 (3.4)	1 (3.4)	1 (3.4)	2 (6.9)	3 (10.3)	2 (6.9)
Guinea pig	22	7 (31.8)	3 (13.6)		6 (27.3)	1 (4.5)	5 (22.7)		
Lizard	7	4 (57.1)			3 (42.9)				
Chinchilla	6	2 (33.3)					4 (66.7)		
Turtle	4	2 (50)	1 (25)		1 (25)				
Squirrel	2	1 (50)						1 (50)	
Bat	1	1 (100)							
Rat	1				1 (100)				
Snake	1				1 (100)				

[a] blood, liquor and other non-urine body fluids

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Table 2

Animal species	*S. aureus*										
	All infected sites			Wounds		Auditory channel		Dermatitis		Other sites	
	n	MR (%)	MS (%)	MR (%)	MS (%)	MR (%)	MS (%)	MR (%)	MS (%)	MR (%)	MS (%)
All species	61	27 (44.3)	34 (55.7)	21 (34.4)	17 (27.9)	2 (3.3)	7 (11.5)	3 (4.9)	3 (4.9)	1 (1.6)	7 (11.5)
Dog	32	18 (56.2)	14 (43.8)	15 (46.9)	6 (18.8)	1 (3.1)	4 (12.5)	1 (3.1)	2 (6.3)	1[a] (3.1)	2[b] (6.3)
Cat	11	4 (36.4)	7 (63.6)	3 (27.3)	1 (9.1)	1 (9.1)	2 (18.2)		1 (9.1)		3[c] (27.3)
Bird	7	1 (14.3)	6 (85.7)	1[e] (14.3)	5 (71.4)						1[f] (14.3)
Rabbit	2	1 (50)	1 (50)	1 (50)	1 (50)						
Guinea pig	4	1 (25)	3 (75)		2 (50)			1 (25)			1[d] (25)
Chinchilla	2		2 (100)		1 (50)		1 (50)				
Turtle	1	1 (100)						1 (100)			
Squirrel	1		1 (100)		1 (100)						
Bat	1	1 (100)		1 (100)							

Abbreviations: n: number; MR: Methicillin-resistant; MS: Methicillin-susceptible

[a]Cystitis; [b] Sinusitis, Pharyngitis; [c]Rhinitis, Cystitis, Sinusitis; [d]Stomatitis [e] Osteomyelitis; [f] Eye infection.

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Table 3:

Date	Host	Source	PFGE[a]	SCC*mec*[b]	PVL[c]
29.04.2003	dog	wound	B	n.t.	negative
07.07.2003	dog	wound	B	n.t.	negative
08.07.2003	dog	wound	A	IV	negative
29.07.2003	dog	otitis	A	IV	negative
23.03.2004	guinea pig	dermatitis	A-1	IV	negative
07.04.2004	dog	wound	A	IV	negative
15.04.2004	dog	wound	A	IV	negative
27.04.2004	cat	wound	A	IV	negative
29.04.2004	dog	dermatitis	A	IV	negative
30.04.2004	dog	wound	A	IV	negative
30.04.2004	dog	wound	A	IV	negative
12.05.2004	cat	wound	A	IV	negative
12.05.2004	dog	cystitis	A	IV	negative
17.05.2004	cat	otitis	BE	IV	negative
15.07.2004	rabbit	wound	A	IV	negative
22.07.2004	dog	wound	BE-1	IV	negative
23.08.2004	dog	wound	A	IV	negative
24.08.2004	dog	wound	A	IV	negative
07.09.2004	dog	wound	A	IV	negative
20.10.2004	dog	wound	A	IV	negative
02.11.2004	cat	wound	A	IV	negative
02.11.2004	parrot	osteomyelitis	A	IV	negative
08.11.2004	bat	wound	A	IV	negative
11.11.2004	dog	wound	A	IV	negative
16.11.2004	dog	wound	A	IV	negative
18.11.2004	turtle	dermatitis	A	IV	negative
22.11.2004	dog	wound	A	IV	negative

Abbreviations:

n.t.: not typeable

A- 1: PFGE type assigned to be closely related to PFP "A"

BE: Human epidemic strain Barnim (Germany)

BE-1: PFGE type assigned to be closely related to Human epidemic strain Barnim (Germany)

[a] PFGE profiles (and subtypes) named with capital letters: A, A-1, B, BE, BE-1

[b] SCC*mec*: staphylococcal cassette chromosome *mec*

[c] PCR result for the Panton- Valentine leukozidin factor

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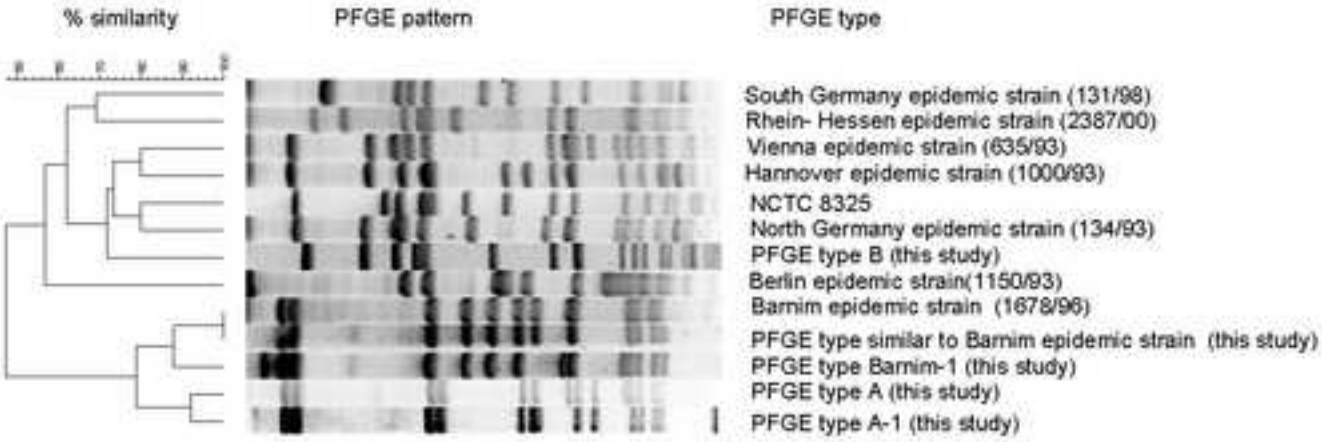

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