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► **To cite this version:**

Birgit Walther, Lothar H. Wieler, Alexander W. Friedrich, Anne-Merethe Hanssen, Barbara Kohn, et al.. Methicillin-resistant (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Veterinary Microbiology*, 2007, 127 (1-2), pp.171. 10.1016/j.vetmic.2007.07.018 . hal-00532295

HAL Id: hal-00532295

<https://hal.science/hal-00532295>

Submitted on 4 Nov 2010

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Accepted Manuscript

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

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PII: S0378-1135(07)00362-8
DOI: doi:10.1016/j.vetmic.2007.07.018
Reference: VETMIC 3768

To appear in: *VETMIC*

Received date: 13-3-2007
Revised date: 16-7-2007
Accepted date: 20-7-2007

Please cite this article as: Walther, B., Wieler, L.H., Friedrich, A.W., Hanssen, A.-M., Kohn, B., Brunenberg, L., Lübke-Becker, A., Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.07.018

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1 **Short communication**

2

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

5

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1 **Abstract**

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

9 A total of 61 *S.aureus* isolates were found during a 20-month period of investigation,
10 27 (44.3%) of them harbouring the *mecA* gene for methicillin-resistance. The majority
11 of MRSA were isolated in specimens from dogs (n=18) and cats (n=4). One guinea
12 pig and one rabbit were found to be positive for an MRSA infected site. Similarly,
13 three exotic animals, a turtle, a bat and a parrot, were found to be infected with
14 MRSA. PFGE and MLST analysis revealed a certain genotype ("A", and "A-1")
15 dominating the isolate collection (23 of 27). Furthermore, one isolate showed
16 homologous PFGE pattern to the German epidemic strain Barnim ("BE") and another
17 one ("BE-1") was considered to be closely related. A third genotype ("B") was
18 detected in two cases. Two different sequence types (ST) were identified among the
19 27 MRSA isolates. PFGE type "A" and both strains related to the Barnim epidemic
20 strain were assigned to ST22, whereas ST239 was associated to PFGE profile "B".
21 The present data show that certain MRSA genotypes are capable of infecting a wide
22 spectrum of small and exotic animals, especially in clinical facilities.

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1 Introduction

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

8 Since the mid 1970`s, cases of infections due to MRSA were reported in different
9 animal species (Devriese and Hommez, 1975; Hartmann et al., 1997; Pak et al.,
10 1999; Shimizu et al., 1997). Meanwhile, MRSA infections in domestic animals have
11 been reported among horses, pigs, cattle, sheep, cats, dogs and rabbits. In the last
12 years however, there has been an increase in reports on MRSA infections in small
13 animals and horses (Middleton et al., 2005; Rich and Roberts, 2006; Seguin et al.,
14 1999; Walther et al., 2006). Moreover, occurrence of MRSA has been reported as a
15 problem in veterinary (teaching) facilities (O'Mahony et al., 2005; Strommenger et al.,
16 2006; Weese et al., 2006).

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

26

1 **Materials and methods**

2 Setting

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

7

8 Bacterial isolation and identification

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

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

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

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

7

8

9 Typing of MRSA strains

10 **PFGE**

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

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

22

23 **MLST**

24 MLST typing of seven housekeeping genes was carried out for all different PFGE
25 types (n= five types: A, A1, B, BE, BE-1) as described elsewhere (Enright et al.,
26 2002).

1 **SCCmec**

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

4

5 **Detection of PVL genes**

6 PCR detection of Panton-Valentine leukocidin (PVL) genes (*lukS-PV-lukF-PV*) was
7 carried out as previously described by Lina et al., 1999.

8

9 **Results**

10 During the 20 month investigation period from May 2003 to December 2004 we
11 screened 869 clinical specimens, which had been delivered to the IMT
12 microbiological laboratory for routine diagnostic purposes. The proportion of
13 specimens for each animal species varied extensively. The absolute majority of
14 specimens were from dogs (67.8%), followed by specimens from cats (17%), birds
15 (6.8%), rabbits (3.3%), guinea pigs (2.5%) and other small and exotic animals. All
16 data including source of animal specimens and body site of origin of all 869
17 specimens are provided in table 1.

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

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

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

7 Most of the MRSA isolates were obtained from wound infections (21 of 27; 77.8%).
8 The infection rate due to MRSA in specimens from wound infections of dogs was
9 7.5% (15 of 201 specimens), in cats 10% (3 of 30 specimens) and in birds 2.3% (1 of
10 43). Other animals were not included in the calculation of wound infection rate due to
11 the small number of wound infections (<20).

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

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

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

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

5 *SCCmec*- typing revealed that PFGE type "A", "A-1", "BE" and "BE-1" was
6 associated to *SCCmec* IV, whereas the *SCCmec* multiplex PCR for the PFGE profile
7 "B" presented no definite results. The PCR showed a positive amplification signal for
8 the loci C and H (related to *SCCmec* type IIIa). However, a positive signal for locus A
9 (related to *SCCmec* type I) was also detected (Oliveira and de Lencastre, 2002).

10 None of the MRSA isolates in the present study harbored the PVL genes.

11

12 **Discussion**

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

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

22 Remarkably, we did not only find MRSA from infected sites of small animals like
23 dogs, cats, guinea pigs and rabbits, as has been reported by others (Baptiste et al.,
24 2005; Boag et al., 2004; Strommenger et al., 2006; Weese, 2005), but also in more
25 exotic animals like turtle, parrot and bat. A case of MRSA infection in a parrot has

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

3 We found a frequency of *S. aureus* in clinical specimens from dogs of 5.4% and of
4 7.4% for cats. These findings support the estimation, that the *S. aureus* frequency
5 rate in clinical specimens from dogs and cats is typically below 10% (Leonard and
6 Markey, 2007).

7 Without regard to animal species, MRSA infections were absolutely predominant in
8 wound infections (77.7%) in this study. O'Mahony and coworkers (O'Mahony et al.,
9 2005) reported a similar rate (77%) for non-equine animals.

10 The MRSA isolates collected in this study underwent different common typing
11 procedures. PFGE analysis revealed, that 22 (+1 subtype: "A-1") of all 27 MRSA
12 isolates could be assigned to the same PFGE type ("A"), including those from the
13 three exotic animals mentioned. Thus, type "A", assigned to ST22, represents 85.2%
14 of all MRSA isolates collected during the present study. Other studies have reported
15 the frequent occurrence of MRSA isolates with a ST22 background in Northern
16 Germany (Witte et al., 2001) human hospitals as well as in other university veterinary
17 settings (Strommenger et al., 2006) for small animals. Furthermore, we have shown
18 evidence for the occurrence of the human epidemic strain Barnim ("BE") as well as
19 for a closely-related subtype designed as Barnim-1 ("BE-1") in animals (both: ST22).

20 The first one was isolated from an auditory channel swab specimen from a cat, and
21 the second one was detected in a wound specimen from a dog (Tab.2; fig.1). These
22 findings support the theory on potential transferability of certain MRSA genotypes
23 between animals and humans (Strommenger et al., 2006).

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

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

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

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

11 It has recently been shown that *S. aureus* strains with invasive potential to humans
12 and pets have arisen from the same genetic background (van Leeuwen et al., 2005).

13 Furthermore, it is well known that the *SCCmec* complex, which harbours the
14 resistance gene *mecA*, has a potential for being transferred horizontally (Enright et
15 al., 2002).

16 As a conclusion of the present study it can be assumed that a single MRSA
17 genotype, apparently well adapted to the local environmental conditions, appeared
18 as a nosocomial pathogen in a small animal clinical facility, regardless of the animal
19 species. Hospital acquired infections due to a single genotype have been reported
20 earlier from veterinary settings (Leonard et al., 2006; Strommenger et al., 2006) and
21 transmissions between man and animal seem to be likely (Baptiste et al., 2005;
22 Manian, 2003; van Duijkeren et al., 2004), but environmental factors (Weese et al.,
23 2004), also play a role in veterinary facilities.

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

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

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

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

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

3

4

5 Acknowledgements:

6 We thank Prof. W. Witte, (Robert Koch Institut, Wernigerode, Germany) for providing
7 all epidemic human MRSA strains used in this study.

8

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21

Table 1

Animal species	n	specimen origin:							
		Wounds (%)	Systemic infection ^a (%)	Respiratory system (%)	Skin and mucosa (%)	Urogenital tract (%)	Auditory channel (%)	Gastrointestinal 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

Table 2

Animal species	<i>S. aureus</i>										
	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

^aCystitis; ^b Sinusitis, Pharyngitis; ^cRhinitis, Cystitis, Sinusitis; ^dStomatitis ^e Osteomyelitis; ^f Eye infection.

Table 3:

Date	Host	Source	PFGE ^a	SCC <i>mec</i> ^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 *SCCmec*: staphylococcal cassette chromosome *mec*

^c PCR result for the Panton- Valentine leukozidin factor

