

Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates

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Received 11 October 2005; returned 17 November 2005; revised 29 November 2005; accepted 6 December 2005

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from pet animals were characterized and compared with human isolates from clonal complexes most prevalent in Central Europe.

Methods: *S. aureus* isolates were investigated for their *in vitro* susceptibility to antimicrobial agents by broth microdilution. Resistance genes and the Pantón–Valentine leucocidin gene *lukF-lukS* were identified by PCR. All isolates were characterized by *Sma*I macrorestriction analysis and *spa* typing to assess their genomic relationships. Representative isolates were additionally analysed by multilocus sequence typing and PCR-directed SCC*mec* typing.

Results: All pet isolates carried the resistance genes *mecA* and *erm(C)* and proved to be resistant to β -lactams and MLS_B antibiotics. In addition, all isolates were resistant to fluoroquinolones. None of the pet isolates carried the Pantón–Valentine leucocidin gene *lukF-lukS*. Macrorestriction analysis revealed that the pet MRSA isolates exhibited four closely related *Sma*I fragment patterns. Moreover, all isolates revealed *spa* type t032. Further analysis of representatives of the different PFGE types revealed the presence of multilocus sequence type ST22 in combination with a type IV SCC*mec* element. Thus, molecular typing results were similar for pet strains and human ST22 reference strains.

Conclusions: Based on their strain characteristics, the MRSA isolates from pets investigated in this study closely resembled ST22 MRSA isolates which are widely disseminated in German hospitals. The results of this study indicate that cross-transmission of MRSA between pet animals and humans might have occurred.

Keywords: antimicrobial resistance, *spa* typing, MLST, SCC*mec*

Introduction

Staphylococcus aureus represents a colonizer and pathogen for humans as well as for various animal species. Prevalence of methicillin-resistant *S. aureus* (MRSA) in human medicine has constantly increased in many parts of the world. MRSA isolates, additionally resistant to numerous commonly used antimicrobial agents, represent a major problem in the hospital environment, causing a variety of serious nosocomial infections, which are extremely difficult to treat.¹ Methicillin resistance in *S. aureus* is mediated by the penicillin binding protein PBP2a with low affinity to β -lactam antibiotics. It is encoded by the gene *mecA*,

residing on a large mobile genetic element designated staphylococcal chromosome cassette *mec* (SCC*mec*). Until now, at least five different types of SCC*mec* have been described.^{2,3}

More recently, reports on community-acquired MRSA (CA-MRSA), causing skin and soft tissue infections as well as fatal pneumonia in people without known risk factors for MRSA infections, are a matter of growing concern. Most CA-MRSA possess a type IV or V SCC*mec* element and the Pantón–Valentine leucocidin locus.^{3,4}

There are only limited numbers of reports on MRSA infections and/or colonization in pet animals. However, since companion animals often are in close contact with their owners, the risk of

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Table 1. Characteristics of strains used in this study

Strain	Origin	Resistance phenotype	Resistance genotype	PFGE pattern	<i>spa</i> type	MLST	SCC <i>mec</i> type
04-3095	dog, urine	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032 ^a	ND	ND
04-3097	dog, implant swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A2	t032	ST22 ^b	IV
04-3098	dog, wound swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3099	dog, wound swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3100	cat, ear swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	B2	t032	ST22	IV
04-3101	dog, wound swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A2	t032	ND	ND
04-3103	dog, joint puncture	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ST22	IV
04-3104	dog, wound swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	B1	t032	ND	ND
04-3105	cat, urine	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3106	dog, skin swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3107	dog, skin swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3108	dog, fistula swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3109	dog, joint puncture	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	B1	t032	ND	ND
04-3110	cat, urine	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
04-3111	dog, wound swab	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	B1	t032	ST22	IV
04-3112	dog, urine	PEN, OXA, ERY, CLI, CHL, CIP	<i>mecA</i> , <i>erm(C)</i>	A1	t032	ND	ND
96-1678	reference strain ST22	PEN, OXA, CIP	<i>mecA</i>	similar to B	t032	ST22	IVc
03-2444	reference strain ST22, subclone	PEN, OXA, CIP	<i>mecA</i>	similar to A	t032	ST22	IV

PEN, penicillin G; OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; CHL, chloramphenicol; CIP, ciprofloxacin; ND, not determined.

^at032 repeat pattern according to www.ridom.de: 26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28.

^bST22 allele pattern according to the MLST home page www.mlst.net: 7-6-1-5-8-8-6.

transmission of pathogenic bacteria between animals and humans (or vice versa) must be taken into consideration. Although early studies on host specificity of *S. aureus* indicated the existence of host-adapted biotypes,⁵ one can speculate that pet animals become reservoirs of 'human' MRSA after exposure to infected humans. Such animals might be a continuous hazard to their owners, especially if their owners have an increased disposition to MRSA infection.⁶

In this study, we report on molecular typing of MRSA from pet animals, originating from different kinds of infections. Typing results were compared with those of MRSA frequently prevalent in hospitals and in the community in order to assess the risk of MRSA transmission originating from pets for their owners (or vice versa).

Materials and methods

Bacterial strains

MRSA isolates were collected at the diagnostic laboratory of the Institute for Microbiology and Infectious Diseases at the School of Veterinary Medicine Hanover, Germany. Strains were isolated sporadically from pet animals admitted to the same veterinary hospital for stationary treatment between September 2003 and July 2004 (Table 1). In this veterinary clinic ~20 000 animals are treated (predominantly dogs and cats) including 16 500 ambulant and 3500 stationary patients per year. Human MRSA isolates 96-1678 and 03-2444 were included as ST22 reference strains. Reference strains for the majority of clonal groups, prevalent in Central Europe during recent years, including the recently emerging CA-MRSA, were taken from the strain collection of our laboratory at the National Reference Center for staphylococci. All isolates were cultured on sheep blood agar and confirmed as *S. aureus* by standard procedures. They were subjected to susceptibility testing by broth microdilution according to DIN 58940⁷ using *S. aureus* strains

NCTC 6571, SA403, SA159 and 1309/80 as reference strains for internal quality assurance. Oxacillin-resistant isolates were screened for the *mecA* gene by PCR.⁸

DNA extraction

Staphylococcal genomic DNA was extracted from 2 mL overnight culture with the DNeasy Tissue Kit (Qiagen, Hilden, Germany) using lysostaphin (100 mg/L, Sigma, Taufkirchen, Germany) to achieve bacterial lysis.

Detection of resistance genes and the Pantone–Valentine leucocidin gene *lukF-lukS*

Relevant antibiotic resistance genes were detected in a multiplex PCR approach.⁸ Strains were screened for genes mediating chloramphenicol resistance in a consensus PCR approach, using degenerate primers.⁹ The presence of the Pantone–Valentine leucocidin gene *lukF-lukS* was investigated by PCR, using the primer pair *lukPV* forward (5'-ATCAT-TAGGTA AAAATGCTCTGGACATGATCCA-3') and *lukPV* reverse (5'-GCATCAAGTGTATTGGATAGCAAAAAGC-3') and *S. aureus* 92-0708 as the positive control.

*Sma*I macrorestriction analysis

All isolates were subjected to *Sma*I macrorestriction analysis according to the standardized HARMONY protocol.¹⁰ Band patterns were analysed using the BioNumerics software (Applied Maths, Sint Martens-Latem, Belgium). Similarity values were computed using Dice coefficient and clustering was performed based on unweighted pair group arithmetic averaging.

spa typing and multilocus sequence typing

The polymorphic X-region of the protein A gene (*spa*) was analysed according to Harmsen *et al.*¹¹ The resulting *spa* types were assigned

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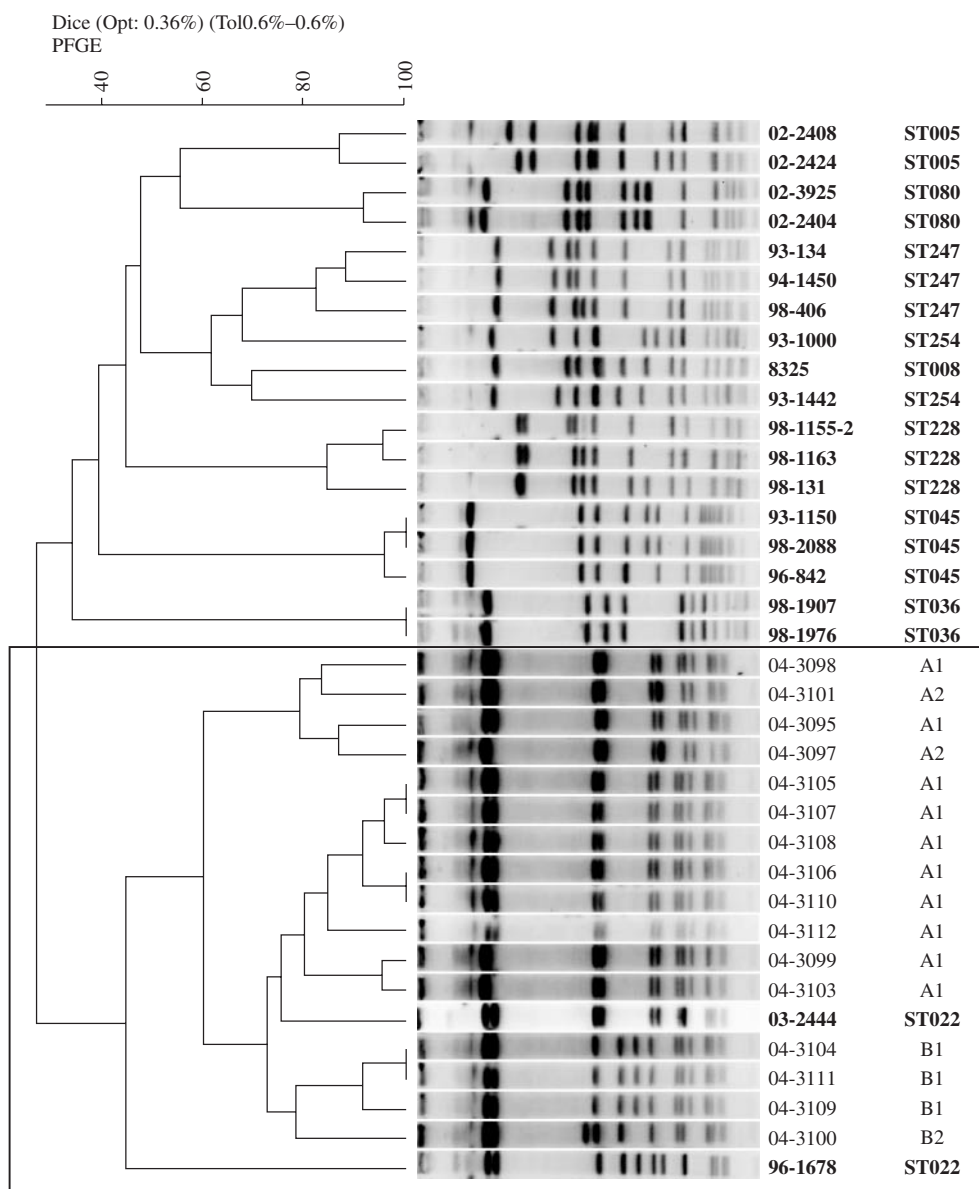


Figure 1. *Sma*I macrorestriction patterns and subsequent cluster analysis for pet-derived MRSA (assigned to four different restriction patterns A1, A2, B1 and B2, respectively) compared with those of reference strains for the most prevalent clonal groups of *S. aureus* in Germany. Reference strains are in bold type and the corresponding MLST for each clonal group is shown.

using the software Ridom StaphType (Ridom GmbH, Würzburg, Germany). Multilocus sequence typing (MLST) was conducted as described previously.¹² Sequencing reactions were carried out using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA). Allele types and resulting sequence types were assigned at the *S. aureus* MLST database via the internet (<http://www.mlst.net>).

Characterization of *SCCmec* by PCR

SCCmec was characterized via a PCR approach, including a combination of different PCR reactions, described previously.¹³ After characterization of the cassette chromosome recombinase complex (*ccrAB*), using the forward primers α 2, α 3 and α 4 (specific for *ccrA1*, *ccrA2* and *ccrA3*, respectively) and the reverse primer β 2 (universally used for *ccrB*¹⁴), primer pairs specific for sequences of particular *SCCmec*

types were used according to Oliveira and de Lencastre.¹³ CIF2F2 and CIF2R2 are located upstream of *ccrA1* in type I *SCCmec*; KDPF1 and KDPR1 anneal upstream of *ccrA2* in type II *SCCmec* which is also characterized by the primer pair IS431P4 and pUB110R1; RIF4F3 and RIF4R9 are located downstream of *mecA* in type III *SCCmec*. For subtyping of type IV *SCCmec* the following primers were used: primers 4a1, 4a2 as well as 4b1 and 4b2 were used to identify the subtypes *SCCmec* IVa and *SCCmec* IVb, respectively,¹⁴ the primers *mecIVc70* and *mecIVc1079* as well as *mecIVd26* and *mecIVd307* were used to distinguish the subtypes *SCCmec* IVc and *SCCmec* IVd.¹⁵

Results and discussion

MRSA infections in pet animals are rarely reported. This is also reflected by the limited number of 16 methicillin-resistant isolates

sporadically collected during a period of ~1 year in the current study. However, recent reports suggest that MRSA in pet animals might be much more prevalent than previously recognized.¹⁶ Thus, pet animals might serve as one putative reservoir for infections and re-infections in humans, as already described in several studies.^{6,17}

All isolates described in this study were confirmed as *S. aureus*, carrying the *mecA* gene (MRSA). Besides *mecA*-mediated resistance to β -lactam antibiotics, the isolates were resistant to MLS_B (macrolide–lincosamide–streptogramin B) compounds mediated by the resistance gene *erm(C)* and to fluoroquinolones (Table 1). The molecular basis for borderline chloramphenicol resistance (all isolates showed MICs of 8–16 mg/L) could not be attributed to known chloramphenicol acetyltransferase genes (*cat*), indicating the presence of an alternative low level chloramphenicol resistance mechanism. All isolates, the human reference strains included, tested negative for the presence of the Pantone–Valentine leucocidin gene *lukF-lukS*.

Upon macrorestriction analysis and subsequent cluster analysis the pet isolates revealed a close relatedness to one another and to ST22 reference strains. Two different restriction patterns, A and B (differing by four to five bands), were distinguished, with most of the isolates exhibiting pattern A. Within the major patterns A and B, minor variations (up to three bands) allowed their differentiation into patterns A1, A2, B1 and B2, respectively (Figure 1 and Table 1). Results were corroborated by *spa* typing with all isolates exhibiting *spa* type t032, representing the type most frequently associated with ST22 isolates (<http://www.ridom.de>). Four isolates, representing the different macrorestriction patterns, were shown to belong to ST22 (Table 1) in MLST analysis. This clone has been rapidly emerging and spreading during the 1990s and is nowadays widely disseminated in hospitals, predominantly in the UK (EMRSA-15¹⁸) and in Germany (Barnim epidemic MRSA¹⁹), where it is mainly disseminated in the northern part of the country. In this area it is restricted to hospitals and not found as CA-MRSA so far. It is striking that the majority of MRSA affected animals in this study originated from the Southern parts of Lower Saxony (a federal state in the north of Germany), where ST22 MRSA is frequently isolated from nosocomial infections indicating a putative transmission between pets and humans. The close relatedness between the animal isolates resembles human MRSA outbreaks in hospitals. Since all MRSA isolates were obtained from infected animals hospitalized at the same veterinary hospital, it may be possible that the pets acquired the MRSA during hospitalization in the veterinary clinic as demonstrated in previous studies.⁶ A recent study revealed evidence of the spread of ST22 EMRSA-15 inside a small animal hospital in the UK, colonizing humans, dogs and inanimate objects also indicating that cross-transmission inside an animal hospital seems to be possible. However, the mode of emergence and spread of this clone remained uncertain.²⁰

PCR analysis of the *SCCmec* element for the pet isolates and reference strain 03-2444 revealed the presence of a *ccrA2* complex in combination with negative PCR results for all *SCCmec* type specific elements indicating the presence of a *SCCmec* type IV element, typical for ST22 isolates. However, in contrast to the ST22 reference strain 96-1678, which is type IVc, subtyping of the type IV element was not successful (Table 1). ST22 MRSA isolates were previously shown to be highly divergent,¹⁸ resulting in a variety of ‘subclones’ characterized by different macrorestriction patterns. This development is also observed in Germany, with the isolate 03-2444 representing one subclone of the ‘classical’ ST22

reference strain 96-1678 (W. Witte, unpublished results). Although a variety of subclones occur in the hospital environment, none of them has been associated with nosocomial outbreaks so far. Most of the pet isolates described herein revealed very similar restriction patterns as compared with reference strain 03-2444 representing a subpopulation of ST22 which might have adapted to pet animals.

In conclusion, pet-animal-derived MRSA isolates originating from different infections belonged to the same clonal lineage as hospital-derived isolates from the same geographic area. This indicates a putative risk of cross-transmission of MRSA between pets and humans. However, due to the limited data about MRSA carriage by owners and veterinary staff, the route of infection can only be speculated upon. Thus, subsequent studies should include pet owners, as well as veterinary hospitals and their staff, in order to trace back the routes of colonization and/or infection and to eradicate putative reservoirs of highly epidemic MRSA clones in pet animals representing a continuous threat for the owners as well as for the animals.

Transparency declarations

None to declare.

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