Methicillin-resistant *Staphylococcus aureus* isolated from dogs and cats in Switzerland

J. R. K. Wipf, V. Perreten

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

**Abstract**

Twenty-two methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from various infected locations in domestic cats and dogs between June 2008 and September 2014 were analyzed for their genotype, genetic fingerprint, virulence and antibiotic resistance profile. Eighteen strains belonged to the clonal complex (CC) 22 [ST22(MLST)-A(PFGE)-t032(*spa*)-IV(SCCmec) and ST22-A-t1214-IV], 2 strains to the livestock-associated MRSA ST398-t011-IV and two were individual strains of ST5-t002-II and ST1-t001-IV. They contained virulence factors such as γ-hemolysins, β-hemolysin converting phage genes, leukocidins and enterotoxins. Most widespread resistances were observed against β-lactams, trimethoprim and fluoroquinolones, but single strains also exhibited resistance to macrolides, lincosamides, aminoglycosides, tetracycline, chloramphenicol and/or mupirocin. The predominant presence of CC22 MRSA strongly indicates clonal spread of a human associated lineage in Swiss companion animals. It is therefore of public health importance to maintain a low level of MRSA infections in animals to avoid uncontrolled dissemination of MRSA clones in humans and animals.

**Keywords:** MRSA, companion animals, *mecA*, infection, genotyping, antibiotic resistance, virulence

**Methicillin-resistente *Staphylococcus aureus* Stämme isoliert aus Hunden und Katzen in der Schweiz**


**Schlüsselwörter:** MRSA, Haustiere, *mecA*, Infektion, Genotypisierung, Antibiotikaresistenz, Virulenz
Originalarbeiten | Original contributions

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Introduction

Staphylococcus aureus can cause a wide range of purulent and toxic infections in humans and animals (Lowy, 1998). Methicillin-resistant Staphylococcus aureus (MRSA) are characterized by the acquisition of staphylococcal cassette chromosome mec (SCCmec) element carrying the mecA gene, a penicillin-binding protein conferring resistance to practically all β-lactams with the exception of ceftaroline (Chambers, 1997; Steed and Rybak, 2010). In addition, MRSA often carries further antibiotic traits which are limiting options for therapy (de Lencastre et al., 2007). During the last decades, MRSA infections have not only been relevant in humans, but also increased in companion animals (David and Daum, 2010; Weese and van Duijkeren, 2010; Vincze et al., 2014b). The most commonly reported MRSA infections in dogs and cats are associated with wounds or surgical sites, the skin, ears and the urinary tract (Weese and van Duijkeren, 2010). Additionally, healthy companion animals can be colonized with MRSA, but prevalence of colonization is significantly lower than in the human population and of transient nature (Weese, 2010). Molecular typing and whole-genome sequencing showed that MRSA strains isolated from household pets are identical to types spreading in the regional human population, suggesting that pets acquire MRSA through their colonized owner (Baptiste et al., 2005; O’Mahony et al., 2005; Moodley et al., 2006; Strommenger et al., 2006; Weese et al., 2006; Loeffler et al., 2013). For instance, clones belonging to the clonal complexes (CC) CC5, CC8, and CC22 are the most frequently isolated strains from canine and feline MRSA colonization and infection in Europe (van Duijkeren et al., 2004; Loeffler et al., 2005; Moodley et al., 2006; Strommenger et al., 2006; Coelho et al., 2011; Monecke et al., 2011; Vincze et al., 2014b). Those MRSA of CC5, CC8, and CC22 belong to the so-called hospital-acquired (HA-MRSA) and community-acquired (CA-MRSA) lineages which spread in human health care settings or communities (Monecke et al., 2011). CA-MRSA have emerged since the mid-1990s and are frequently associated with the acquired Panton-Valentine leucocidin (PVL), a cytotoxin which causes severe skin and soft tissue damages (David and Daum, 2010). CC398 or livestock-associated MRSA (LA-MRSA) which is mainly associated with pigs, horses, farm personnel and veterinarians has also been reported to cause infection in companion animals, but less frequently (Nienhoff et al., 2009; Monecke et al., 2011; Sieber et al., 2011; Haenni et al., 2012; Wettstein et al., 2014; Lekkerkerk et al., 2015).

Since 2008, some of the MRSA isolated from companion animals in Switzerland have been referred to the Centre for Zoonoses, Bacterial Animal Diseases and Antibiotic Resistance (ZOBA) of the Institute of Veterinary Bacteriology, University of Bern, for confirmation and strain collection establishment. These strains were further characterized to determine which types of MRSA are associated with infections in Swiss pets and determine their virulence and resistance profile.

Material and Methods

Sample collection, species identification and growth conditions

A total of 22 MRSA strains were obtained from routine diagnostic procedures from the ZOBA, University of Bern, the IDEXX Diavet Laboratory, Bäch, Switzerland and the Laboratory Laupeneck AG, Bern, Switzerland. Strains could be traced back to nine different veterinary clinics (VC 1 to 9) of different geographical regions of Switzerland (Fig. 1). All strains were isolated from clinical material [purulence, skin lesions (n=12); surgical transplants, intra-operative swabs (n=8); tracheobronchial exudate (n=1); urine (n=1)] from domestic cats (n=9) and dogs (n=13) between June 2008 and September 2014. Isolates were grown aerobically at 37°C overnight on tryptone soy agar plates containing 5% sheep blood (TSA-S; Becton, Dickinson & Company, Franklin Lakes, NJ). Species identification was executed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Microflex LT, Bruker Daltonik GmbH, Bremen, Germany). Methicillin-resistance was confirmed using Vitek 2 with Gram-Positive (ASP-GP69) cards (BioMérieux, Mary l’Etoile, France) following the manufacturer’s instructions, as well as by the detection of the mecA gene by PCR (Schnellmann et al., 2006) and by PBP2a latex agglutination test (Oxoid, Hampshire, United Kingdom).

Antimicrobial susceptibility testing and virulence profile determination

Minimal inhibitory concentrations (MICs) of 19 antibiotics, namely cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, oxacillin, penicillin, rifampicin, streptomycin, sulfamethoxazole, tetracycline, tiamulin, trimethoprim and vancomycin were tested by broth microdilution in Mueller-Hinton Broth using custom-made susceptibility plates NLE-UST1 (TREK Diagnostics Systems, East Grinstead, England). Only antibiotic classes for which MRSA exhibited resistance have been specified in Table 1. Antimicrobial susceptibility was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines http://www.eucast.org,
Table 1: Origin, genetic characteristics and resistance pattern of MRSA isolated from infections in Swiss cats and dogs

<table>
<thead>
<tr>
<th>MLST-spa-SCCmec-PFGE Representative strain</th>
<th>Origin (n)</th>
<th>Virulence genes</th>
<th>Antibiotic resistance profile (breakpoints determining resistance in mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST22-t032-IV-A KM1224/08</td>
<td>1/0</td>
<td>hlgA, hlgB, hlgC, seg, sei, selm, seln, selo, selu</td>
<td>FOX/OXA (≥4/2) PEN (≥0.12) TMP (≥2) TET (≥1/16) GEN/KAN (≥2/0.5) ERY/CLI (≥2/0.5) CHL (≥8) STR (≥16) MUP (≥256) CIP (≥1)</td>
</tr>
<tr>
<td>ST22-t032-IV-A KM1474/08</td>
<td>3/10</td>
<td>hlgA, hlgB, hlgC, seg, sei, selm, selo, selu, sak, chp, scn</td>
<td>mecA blaZ F99K</td>
</tr>
<tr>
<td>ST22-t032-IV-A IMD1271/11</td>
<td>1/0</td>
<td>hlgA, hlgB, hlgC, seg, sei, selm, selo, selu, sak, chp, scn</td>
<td>mecA blaZ F99K</td>
</tr>
<tr>
<td>ST22-t032-IV-A KM894/09</td>
<td>1/0</td>
<td>hlgA, hlgB, hlgC, seg, sei, selm, selo, selu, sak, chp, scn</td>
<td>mecA blaZ</td>
</tr>
<tr>
<td>ST22-t032-IV-A KM1341/12</td>
<td>0/1</td>
<td>hlgA, hlgB, hlgC, seg, sei, selm, selo, selu, sak, chp, scn</td>
<td>mecA blaZ</td>
</tr>
<tr>
<td>ST22-t1214-IV-A IMD244/13</td>
<td>1/0</td>
<td>hlgA, hlgB, hlgC, seg, sei, selm, selo, selu, sak, chp, scn</td>
<td>mecA blaZ F99K</td>
</tr>
<tr>
<td>ST398-t011-IV-D KM438/09</td>
<td>0/1</td>
<td>hlgA, hlgB, hlgC</td>
<td>mecA blaZ dfrK tet(M) aac(6')-le-aph(2')-I, ant(4')-Ia erm(C)</td>
</tr>
<tr>
<td>ST398-t011-IV-D KM1150-1/13</td>
<td>1/0</td>
<td>hlgA, hlgB, hlgC</td>
<td>mecA blaZ dfrK tet(M) aac(6')-le-aph(2')-Ia,str, ant(6)-la</td>
</tr>
<tr>
<td>ST1-t001-IV-B KM1640/14</td>
<td>1/0</td>
<td>hlgA, hlgB, hlgC, sak, chp, scn, lukD, lukE</td>
<td>mecA blaZ tet(K) aph(3')-III, erm(C)</td>
</tr>
<tr>
<td>ST5-t002-II-C IMD39/12</td>
<td>0/1</td>
<td>hlgA, hlgB, hlgC, sed, seg, sei, selm, selo, selu, sak, chp, scn, lukD, lukE</td>
<td>mecA blaZ ant(4')-la,erm(A) cat,C221</td>
</tr>
</tbody>
</table>

FOX, cefoxitin; OXA, oxacillin; PEN, penicillin; TMP, trimethoprim; TET, tetracycline; GEN, gentamicin; KAN, kanamycin; ERY, erythromycin; CLI, clindamycin; CHL, chloramphenicol; STR, streptomycin; MUP, mupirocin; CIP, ciprofloxacin. Antibiotic resistance genes and their function: mecA, methicillin-resistance gene encoding PBP2a for resistance to all β-lactam-antibiotics; blaZ, β-lactamase gene; dfrK, acquired dihydrofolate reductase; Dhfr, chromosomal dihydrofolate reductase; tet(K), tetracycline efflux gene; tet(M), ribosome protection tetracycline resistance gene; aac(6')-le-aph(2')-Ia, aminglycoside acetyltransferase and phosphotransferase tandem genes; ant(4')-Ia, amikacin and tobramycin nucleotidytransferase gene; aph(3')-III, aminoglycoside phosphotransferase gene; ant(6)-Ia, streptomycin nucleotidytransferase gene; erm(A) erm(C), macrolide, lincosamide and streptogramin B 23S rRNA methylase genes; cat,C221, chloramphenicol acetyltransferase gene; str, streptomycin adenyltransferase gene; lars, bacterial isoleucyl-tRNA synthetase; GrLA, topoisomerase IV; GryA, topoisomerase II. Virulence genes and their functions: hlgA hlgB hlgC, γ-hemolysin genes; sec sed seg sei selm selo selu, enterotoxin genes; sak chp scn, β-hemolysin converting phage genes; lukD lukE, leukocidin genes.

MIC breakpoints that determine resistance were recommended from EUCAST for staphylococci (www.eucast.org). Resistance breakpoints for streptomycin and kanamycin were those recommended by the French Society for Microbiology (www.sfm-microbiologie.org).
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except for the breakpoints of streptomycin and kanamycin which were obtained from the French Society for Microbiology (www.sfm-microbiologie.org), and those of sulfamethoxazole obtained from the CLSI guidelines (CLSI, 2014). Due to the lack of resistance breakpoint for the pleuromutilin tiamulin, resistance was evaluated based on decreased susceptibility and detection of a tiamulin resistance gene \([cfr, vga]\) (Long et al., 2006; Schwendener et al., 2011). Antibiotic resistance genes were detected with a custom-made microarray (AMR+ ve-5 array tubes; Alere GmbH, Jena, Germany) (Straus et al., 2015). Mutations in the quinolone resistance determining region (QRDR) of the GyrA and GrlA topoisomerase subunits, as well as in the mupirocin resistance region of the isoleucyl-tRNA synthetase were identified by amino acid sequence analysis of translated PCR products as described previously (Schmitz et al., 1998; Fujimura et al., 2003). Mutations affecting trimethoprim susceptibility of \(S. aureus\) were detected by amino acid sequence analysis obtained from the entire dihydrofolate reductase gene of \(S. aureus\) amplified by PCR using primers \(dhfr-F\) (5’- AGGAATTACATGAATGTTGTTGTTGCTTC) and \(dhfr-R\) (5’- GCAAAATCATTATTCTATCACACTTTATG) (Vickers et al., 2009).

Genotyping

Genetic relatedness of the MRSA strains was determined by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), \(spa\) typing and SCCmec typing. PFGE was performed on DNA digested with restriction enzymes Smal or Cfr91 for strains KM438/09 and KM1150/13, the latter enzyme was used only when methylation of the Smal restriction site was observed. Runs were performed on a CHEF DR III apparatus (Bio-Rad, Hercules, CA, USA) according to the Centers for Disease Control and Prevention (CDC) Laboratory Protocol for Molecular Typing of \(S. aureus\) by PFGE (http://www.cdc.gov/hai/pdfs/labsettings/ar_mras_pfge_s_aureus.pdf). Restriction fragment migration profiles were compared using BioNumerics 7.1 (Applied Maths, Kortrijk, Belgium). Sequence types (ST) and \(spa\)

![Dendrogram of DNA fingerprint patterns of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from infection sites of Swiss cats and dogs by Smal- and Cfr91-PFGE, as well as origin, host-species and isolation site of MRSA strains. Cluster analysis was performed with BioNumerics 7.5 (Applied Maths, Kortrijk, Belgium) using the Dice correlation coefficient and the unweighted pair group mathematical average (UPGMA) clustering algorithm with 0.5% optimization and 1% band tolerance.](Fig. 1: Dendrogram of DNA fingerprint patterns of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from infection sites of Swiss cats and dogs by Smal- and Cfr91-PFGE, as well as origin, host-species and isolation site of MRSA strains. Cluster analysis was performed with BioNumerics 7.5 (Applied Maths, Kortrijk, Belgium) using the Dice correlation coefficient and the unweighted pair group mathematical average (UPGMA) clustering algorithm with 0.5% optimization and 1% band tolerance.)
types were determined as described previously (Enright et al., 2003; Harmsen et al., 2003) and assigned using the MLST-Homepage (http://saureus.mlst.net) and the Ridom StaphType software (Ridom StaphType, RidomGmbH, Würzburg, Germany), respectively. SCCmec types were determined using a multiplex PCR assay (Kondo et al., 2007).

**Results**

MRSA were isolated from surgical intervention sites, skin infections/purulences or the urinary and pulmonary tract of dogs and cats from different veterinary clinics in Switzerland (Fig. 1). Four different clonal complexes were found (CC1, CC5, CC22, CC398). MRSA of CC22 were the most frequent, with 18 strains detected in 6 different veterinary clinics (Fig. 1). All the strains belonging to CC22 also displayed a similar PFGE pattern gathering into PFGE cluster A (Fig. 1). Fourteen strains even exhibited the exact same PFGE profile. Differences in the PFGE profiles of the remaining 4 CC22 strains KM1224/08, IMD1271/11, KM894/09 and KM1341/12 resulted from variations in 2 to 4 fragments in the PFGE pattern (Fig. 1). The strains also diverged from those of the larger CC22 cluster by containing less or additional virulence or resistance genes (Tab. 1). Otherwise, all CC22 strains contained the virulence factors hlgA, hlgB (lukF), hlgC (lukS), which are γ-hemolysin associated genes, the enterotoxin genes sej, sei, selm, seln, selo and selu, as well as the β-hemolysin converting phase genes sak, chp and sna. Furthermore, this larger group of CC22 strains exhibited the same resistance pattern being resistant to the β-lactams penicillin ( mecA, blaZ ), oxacillin and cefoxitin [ mecA ]

Molecular typing of MRSA recovered from infection sites of Swiss companion animals identified clonal lineages similar to those associated with MRSA colonization and infection in companion animals in Europe. CC22 MRSA is the most frequent clonal lineage causing infection in companion animals in Switzerland and among the most predominant lineages in pets from Germany, Portugal, Ireland, the UK and Sweden (van Duijkeren et al., 2004; Loeffler et al., 2005; Moodley et al., 2006; Coelho et al., 2011; Grönland Andersson et al., 2014; Vincze et al., 2014b). CC22 MRSA belong to HA-MRSA pandemic in humans all over the world and the specific ST22-IV MRSA clone is the second most isolated lineage reported in human hospitals in Switzerland (Monecke et al., 2011; Senn et al., 2013). Pets may acquire such HA-MRSA or CA-MRSA through their owners or caretakers, but colonization is usually of transient nature (Weese, 2010; Weese and van Duijkeren, 2010; Loeffler et al., 2013). In cases of surgical interventions, accidental wounding or skin diseases, this transient colonization period highly increases the risk of infection development in animals. Furthermore, isolation of a CC22 clone with the exact same fingerprint, virulence and resistance profile over a long time period in veterinary clinics and practices of different regions in Switzerland suggests that these CC22 clones can persist and disseminate in the local veterinary environment. Admission to veterinary clinics, especially if they have more than 10 employees, antimicrobial courses and having received surgical implants, are among the reported risk factors of MRSA colonization and infection development (Loeffler and Lloyd, 2010; Soares Magalhaes et al., 2010; Vincze et al., 2014a). Whether animal owners or the veterinary setting play a role as a source for MRSA infection in dogs and cats in Switzerland was not investigated in our study. However, a study conducted in 2012 on the prevalence of MRSA in Swiss veterinarians revealed that small animal practitioners were colonized with MRSA clones CC398, CC5, CC8 and CC88 lineages, but no CC22 clones were detected at this time (Wettstein et al., 2014). The two CC398 LA-MRSA from pets in this study exhibited the exact same spa-type, resistance gene pattern and virulence gene pattern as the MRSA ST398-t011-IV lineages previously isolated from Swiss large animal veterinarians, horse clinic personnel and horses indicating the broad potential for dissemination of this clonal lineage in the

**Discussion**

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veterinary setting (Huber et al., 2010; Sieber et al., 2011; Wettstein et al., 2014). ST3-t002-II MRSA (IMD39/12) of which we only had a single isolate was already detected in Swiss small animal practitioners in 2012 and is a worldwide pandemic clone and the most prevalent clone in Swiss hospitals (Monecke et al., 2011; Senn et al., 2013; Wettstein et al., 2014). The presence of human MRSA clones like those of CC22, CC5 and CC1 in small companion animals is of public health concern and emphasizes the need for infection control in both human and veterinary settings.

**Conclusion**

This study shows that Swiss cats and dogs may get infected by MRSA which are resistant to clinically important antibiotics and belong to known clonal lineages with large potential for nosocomial dissemination in both animal and human hospitals. It is therefore necessary to maintain a low level of MRSA infection in companion animals keeping continuous and strict infection control strategies following guidelines for prudent use of antimicrobials, such as the guidelines recently released by the European Union (European Commission, 2015).

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*Souches de Staphylococcus aureus résistantes à la méthicilline isolées chez des chiens et des chats en Suisse*

Entre juin 2008 et septembre 2014, 220 souches de *Staphylococcus aureus* résistantes à la méthicilline (SARM) ont été isolées de divers foyers infectieux chez des chiens et des chats et leur génotype, leur virotype et leur profil de résistance aux antibiotiques ont été déterminés. Le complexe clonal (CC) 22 [ST22(MLST)-A(PFGE)-t032(spa)-IV(SCCmec) et ST22-A-t1214-IV] était le type de SARM principal, suivi par le CC398 (ST398-D-t011-IV) normalement associé aux animaux de rente ainsi que par quelques autres souche de SARM (ST5-C-t002-II, ST1-B-t001-IV). Les types de SARM de cette étude avaient un type de virulence relativement conservé, marqué par des gènes associés à la γ-hémolysine, des gènes d’entérotoxines, des gènes de virulence sur les phages convertissant la β-hémolysin et des gènes de leucocidine. Les souches étaient en outre principalement résistantes au β-lactame, au triméthoprime et aux fluoroquinolones, certaines d’entre elles présentant des résistances supplémentaires aux macrolides, aux lincosamides, aux aminoglycosides, aux tétracyclines, au chloramphénicol et/ou à la mupirocine. La fréquence des infections avec des SARM du complexe clonal CC22 indique clairement que ce clone associé à l’être humain peut se répandre facilement dans la population suisse d’animaux de compagnie. Pour réduire cette expansion de SARM chez les hommes et les animaux, il faut chercher à limiter le plus possible les infections dues aux SARM chez les animaux de compagnie.

**Ceppi di Staphylococcus aureus, resistenti alla meticillina, isolati in cani e gatti in Svizzera**

Ventidue ceppi di *Staphylococcus aureus* (MRSA) resistenti alla meticillina sono stati isolati tra giugno 2008 e settembre 2014 in diverse aree d’infezione in cani e gatti. Si è potuto determinare il loro genotipo, virotipo e profilo di resistenza agli antibiotici. Il complesso clonale (CC) 22 [ST22 (MLST)-A(PFGE)-t032 (spa)-IV (SCCmec) e ST22-A-t1214-IV] era il tipo di MRSA predominante, seguito dal clone CC398 associato agli animali da reddito (ST398-D-t011-IV) e da ceppi occasionali di altri tipi di MRSA (ST5-C-t002-II, ST1-B-t001-IV). I tipi di MRSA di questo studio avevano un tipo di virulenza relativamente stabile, marcatto da geni associati a γ-emolisina, enterotossigeni, geni virulenti a fagi di conversione alla beta-emolisina e geni con leucocidine. I ceppi erano prevalentemente resistenti a β-lattamici, trimetoprima e fluoroquinoloni, anche se ceppi singoli mostravano una resistenza supplementare a macrolidi, lincosamidi, aminoglicosidi, tetracicline, cloramfenico e/o mupirocina. La frequenza delle infezioni da MRSA CC22 suggerisce fortemente che questo clone associato agli umani possa facilmente diffondersi nella popolazione dei piccoli animali in Svizzera. Al fine di contenere la diffusione di MRSA nell’uomo e negli animali, l’insorgenza di infezioni MRSA in piccoli animali deve essere mantenuta a un basso livello.
References


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Corresponding address

Prof. Dr. Vincent Perreten
Institute of Veterinary Bacteriology
Vetsuisse Faculty
University of Bern
Länggassstrasse 122
CH-3001 Bern
Phone: +41 (0)31 631 2430
Fax: +41 (0)31 631 2634
E-Mail: vincent.perreten@vetsuisse.unibe.ch