

Clinical, microbiological, and molecular characterization of methicillin-resistant *Staphylococcus aureus* infections of cats

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Objective—To compare clinical information obtained from medical records of cats with methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S aureus* (MSSA) infections, evaluate antibiograms of MRSA and MSSA for multiple-drug resistance (MDR), and characterize the strain type and staphylococcal chromosome cassette (SCC)*mec* type of each MRSA.

Sample Population—70 *S aureus* isolates obtained from 46 cats.

Procedures—Clinical information obtained from medical records, including signalment, clinical signs, histologic examination of affected tissues, and outcomes, was compared between the 2 groups. Composite antibiograms of MRSA and MSSA were compared statistically. The MRSA strains were characterized by use of pulsed-field gel electrophoresis and SCC*mec* typing.

Results—No statistical differences in signalment or subjective differences in clinical signs or outcomes were detected between groups with MRSA or MSSA infection. Significant differences in antimicrobial resistance were detected, with MRSA having complete resistance to fluoroquinolone and macrolide antimicrobials, whereas MSSA maintained a high frequency of susceptibility. Seven pulsed-field patterns were observed in 15 MRSA strains; all but 1 were highly related. All MRSA isolates contained a type II SCC*mec* element.

Conclusions and Clinical Relevance—Because MDR cannot be predicted in staphylococcal infections in cats on the basis of clinical signalment, culture and susceptibility testing are recommended whenever initial empirical treatment is unsuccessful. Molecular characterization of MRSA strains suggests that there has been reverse-zoonotic transmission from humans.

Impact for Human Medicine—The SCC*mec* type II element is typically associated with nosocomial MRSA infections of people. Cats may serve as reservoirs for MRSA infections in humans. (*Am J Vet Res* 2006;67:1421–1425)

Bacteria of the genus *Staphylococcus* are members of the commensal cutaneous and mucosal microflora of cats. Coagulase-positive species, such as *Staphylococcus intermedius*, *Staphylococcus hyicus*, and

ABBREVIATIONS

MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-susceptible <i>S aureus</i>
PFGE	Pulsed-field gel electrophoresis
PFP	Pulsed-field pattern
SCC	Staphylococcal chromosome cassette
MDR	Multiple-drug resistance
PBP	Penicillin-binding protein

Staphylococcus aureus, and several coagulase-negative species have been isolated in microbiological surveys of skin and mucous membranes in healthy cats.¹⁻⁷ Because household cats are more frequently colonized by coagulase-positive staphylococci than are those living in cat-teries, transmission from humans has been suggested.¹

When cutaneous or systemic disease disrupts defense mechanisms of the skin's surface, bacterial infection (pyoderma) or otitis externa may result from these same staphylococcal species. Invasive infections involving the genitourinary tract, respiratory tract, joints, and body cavities may also result by ascension along epithelial tracts, introduction via penetrating wounds, or hematogenous spread. The coagulase-positive species are the most common causes of staphylococcal infections in cats, and information is lacking regarding the pathogenicity of coagulase-negative staphylococci. However, coagulase-negative staphylococci are receiving renewed attention with regard to their medical importance in humans and cats.^{8,9}

Isolates of MRSA are associated with nosocomial and community-acquired infections in humans, and some strains may cause more severe clinical signs than are seen with infections attributable to MSSA isolates.¹⁰ Domestic animals, including cats, are reportedly also susceptible to MRSA infections, and concerns that companion animals may serve as reservoirs of MRSA have been emphasized in several reports.¹¹⁻¹³ To our knowledge, clinical descriptions of MRSA infection or analysis of the microbiological and molecular characteristics of a large series of MRSA isolates obtained from cats has not been reported.

The purposes of the study reported here were to describe the clinical, microbiological, and molecular characteristics of MRSA infections of cats. We hypothesized that MRSA infections of cats would cause more severe clinical signs than would be seen with MSSA infections, such as deeper tissue invasion, increased degree of tissue injury, and increased morbidity and mortality rates. We also hypothesized that genetic analysis of isolates obtained from cats would reveal

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them to be consistent with the most common strains that cause community-acquired infections in humans.

Materials and Methods

Sample population—Isolates of *S aureus* obtained from cats were used for the study. A computerized search was performed of all *S aureus* isolates obtained from cats and identified by the clinical microbiology laboratory at our facility from September 1, 2002, to August 30, 2005.

Microbiologic analysis—Identification of bacterial species and antimicrobial susceptibility patterns (antibiograms) was generated by use of an automated system.^a Antimicrobial agents were selected for routine testing as described by the Clinical Laboratory Standards Institute.^b Oxacillin was used as a substitute for methicillin in susceptibility tests. Susceptibility to enrofloxacin and marbofloxacin was determined by use of Kirby-Bauer disk diffusion. For some cats, multiple *S aureus* isolates were obtained during the course of treatment. In all cases, only the antibiogram data obtained from the initial isolate were used for evaluation. Composite antibiograms were then calculated for MRSA and MSSA isolates and compared statistically.

Clinical information—Data on signalment of affected cats were recorded and compared with that of the hospital population of cats for the study period. Data for 8 cats were not included in any of the clinical analyses because samples from these cats were obtained by referring practitioners and specimens were mailed to our laboratory without a complete medical history or clinical information. The origin of each *S aureus* isolate was catalogued on the basis of body site. Characteristics of each infection, including histologic features (when available), response to antimicrobial treatment, and final outcome, were evaluated.

PFGE analysis—The PFGE analysis was performed on all available MRSA isolates, as described elsewhere.¹⁴ Commercially available software^c was used to determine the percentage of similarities identified on a dendrogram derived from the unweighted pair-group method by use of arithmetic means and based on Dice coefficients. A similarity coefficient of 80% was selected to define PFP clusters described for *S aureus*.¹⁵

Multiplex PCR assay for SCCmec typing—The SCCmec typing was performed as described elsewhere,¹⁶ with a few modifications. The DNA was extracted by use of a DNA purification kit,^d with lysostaphin and lysozyme at concentrations of 10 mg/mL and RNAase at a concentration of 3 mg/mL for cell lysis. The PCR amplifications were performed in a final volume of 50 μ L on a gradient thermal cycler.^e

Statistical analysis—To determine differences in susceptibility to antimicrobials between MRSA and MSSA, the Fisher exact test was used. The Bonferroni correction was applied to adjust for multiple comparisons. Values of $P < 0.006$ were considered significant. To assess differences between the hospital and study population with regard to signalment, the Fisher exact test was used for sex and the t test was used for age. Data were analyzed by use of commercial software packages.^{f,g} Data were reported as frequencies and percentages or means with ranges for categorical and continuous data, respectively.

Results

Sample population—During the 36-month period, 70 *S aureus* isolates were obtained from 46 cats. Multiple isolates were obtained from multiple body sites or at various time points from each of 17 cats.

Each isolate from a common source was included in the molecular analysis only when it had a unique antibiogram. Sixty-four isolates had unique antibiograms, and of these, 17 (26.6%) were resistant to oxacillin. Significant differences in susceptibility to antimicrobials were detected between MRSA and MSSA. Most notably, all MRSA strains were resistant to all fluoroquinolone and macrolide antimicrobials, whereas MSSA strains maintained a high frequency of susceptibility to these 2 classes as well as to β -lactam antimicrobials (Table 1).

Clinical information—Signalment data were analyzed for 38 of 46 cats. There were 26 male and 12 female cats, whereas the total hospital population during this period consisted of 6,502 male and 4,647 female cats. Age range for cats with *S aureus* infections was 0.5 to 18 years (mean, 7.6 years), whereas age range for the total hospital population was 0.1 to 24 years (mean, 6.9 years). There were no significant differences in sex or age distribution between cats with *S aureus* infections and the total hospital population or between the MRSA and MSSA subgroups.

Predisposing diseases resulting in secondary MRSA infections of 11 cats in the study included cutaneous sporotrichosis ($n = 1$), urethral obstruction (5), neoplasia (4), and cholangiohepatitis (1). Primary infection could not be assumed for any of the cats. Predisposing diseases for secondary MSSA infections of 27 cats in the study included skin or ear canal disease ($n = 9$), genitourinary tract disease (4 with urethral obstruction and 3 with chronic renal failure and recurrent lower urinary tract infections), respiratory tract disease (2), neoplasia (5), and cardiovascular disease (2). Two cats with MSSA infection appeared to have primary MSSA infection (1 cat had lymphadenitis [multiple peripheral lymph nodes] and nodular cellulitis, and 1 cat had central vestibular disease). Both of these cats responded to appropriate antimicrobial treatment (as determined by bacterial susceptibility testing), and clinical signs did not recur.

Table 1—Antimicrobial susceptibility of *Staphylococcus aureus* isolates obtained from 46 cats.

Antimicrobial	MRSA isolates (n = 13)*		MSSA isolates (33)*	
	No.	%	No.	%
Trimethoprim-sulfamethoxazole	12	92	32	97
Marbofloxacin†	0	0	32	97
Enrofloxacin†	0	0	32	97
Tetracycline	9	69	31	94
Clindamycin†	0	0	32	97
Erythromycin†	0	0	27	82
Chloramphenicol	10	77	33	100
Gentamicin	12	92	32	97
Oxacillin†	0	0	33	100
Cefazolin†	0	0	33	100
Amoxicillin-clavulanate†	0	0	33	100

*All 13 (100%) MRSA and 26 of 33 (79%) MSSA isolates were β -lactamase producers. †Within an antimicrobial, susceptibility differed significantly ($P < 0.006$) between MRSA and MSSA isolates.

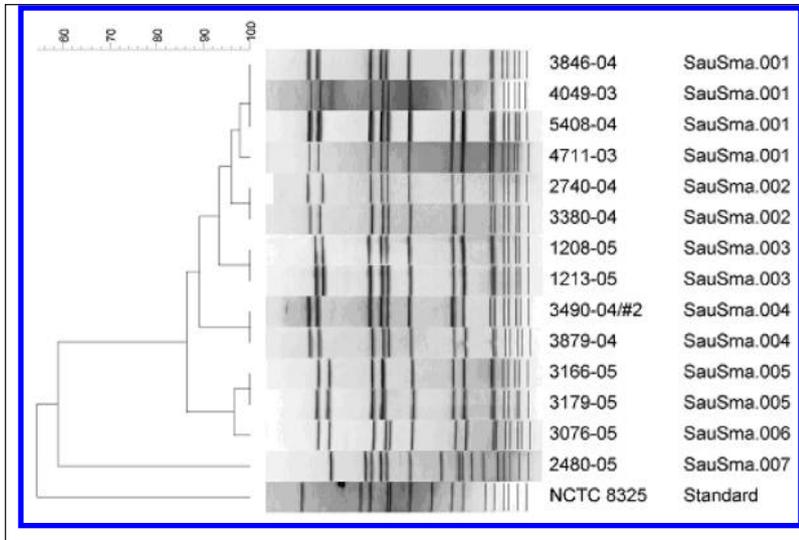


Figure 1—Dendrogram of PFGE patterns obtained for 14 MRSA isolates obtained from cats. The PFPs are indicated as SauSma numbers 1 to 7, which indicate that *S aureus* is the organism and *Sma* is the restriction enzyme used in the analysis. The scale on the upper left side indicates the percentage similarity among isolates. Two PFP clusters are evident (PFPs 1 to 4 and PFPs 5 and 6). Except for strain 2480-05, all PFGE patterns have > 80% similarity, which suggests that these strains are highly clonal.

For 3 cats with multifocal lymphadenitis, surgical biopsy specimens were available for histologic examination. Lymph node tissues had large areas of necrosis with suppurative to pyogranulomatous inflammation and numerous gram-positive cocci. Although eosinophilic inflammation was evident, eosinophils were the minority cell type. Cultures of tissue samples obtained from each of these 3 cats yielded MSSA.

Review of the medical records suggested that none of the cats died as a direct result of staphylococcal infection, nor were there subjective differences in severity of infection or response to appropriate antimicrobial treatment (data not shown). However, data regarding antimicrobial use and final outcome were unavailable for 5 cats with MSSA and 1 cat with MRSA.

PFGE analysis—Fifteen of 17 MRSA isolates had been preserved in cryogenic tubes^h at -80°C and therefore were available for PFGE analysis. Fourteen of these isolates had unique antibiograms, and 7 PFPs were evident. With the exception of strain 2480-05, all isolates were highly related, as determined by the results of PFGE analysis (Figure 1). Two small clusters were evident, with PFPs 1 to 4 identified in 1 cluster and PFPs 5 and 6 identified in another cluster. Multiple isolates from a single cat were identical, regardless of source, date of collection, or antibiogram. For example, strains 1208-05 and 1213-05 were isolated from clinical specimens from a specific cat on separate days but had differing antibiograms. Similarly, strains 3179-05 and 3166-05 were isolated from 2 clinical specimens obtained on the same day from a particular cat, but they had differing antibiograms.

Multiplex PCR assay for SCCmec typing—All 15 MRSA isolates possessed a type II SCCmec element (data not shown).

Discussion

Since the introduction of antibacterial drugs into the practice of medicine, resistant strains of staphylococci have proliferated as a result of antimicrobial selection pressure and MDR has now become common. Currently, a substantial number of staphylococcal species that infect humans and domestic animals have some degree of MDR.^{17,18} In humans, resistance of *S aureus* to methicillin was documented in Europe soon after the antimicrobial was introduced into clinical practice.¹⁹ Methicillin resistance in staphylococci is conferred by an acquired PBP known as PBP2' or PBP2a. This PBP is encoded by the *mecA* gene and confers an intrinsic resistance to all β -lactam antimicrobials and their derivatives.²⁰ The *mecA* gene is carried on a mobile genetic element known as SCCmec, and 5 types of these elements (designated SCCmec types I through V) have been described.²¹

The MRSA can be transmitted among people within hospital settings (hospital-acquired MRSA) and through casual contact within the community (community-acquired MRSA).^{21,22} Infection in domestic animals has received attention in the scientific literature as a reflection of the serious nature of MRSA infections in humans.²³⁻²⁷ The potential for reverse-zoonotic transmission and the creation of animal reservoirs for the reinfection of humans are public health concerns.¹¹⁻¹³

We hypothesized that MRSA infections would affect a different population of cats, cause more severe clinical signs, and carry a less favorable prognosis than would MSSA infections. However, analysis of our results does not indicate a discernible difference in signalment, clinical signs, or outcomes between the 2 groups. Caution may be advisable in interpreting these data because of the relatively low number of MRSA infections and lack of complete records for several cats.

Because some MRSA strains may express virulence factors that MSSA strains do not, MRSA infections of humans may cause clinical signs that are distinguishable from those for MSSA infections. In cats, an association between MRSA infections, subcutaneous abscesses, and lymphadenitis has been reported²⁸ for a series of surgical biopsy specimens. In that study, investigators detected a histologic pattern that included central abscesses with colonies of bacterial cocci surrounded by collagenous granulation tissue and prominent eosinophilic inflammation. In 15 of 17 isolates in that study, gram-positive cocci had positive results for PBP2' when tested by use of an immunohistochemical technique. Lymphadenitis was the predominant clinical sign in 3 cats of the study reported here, 1 of which had multifocal subcutaneous nodules. Although the histologic patterns observed in the lymph nodes of these 3 cats were analogous to those described by investigators in that other study,²⁸ all 3 of our cats were culture-positive for MSSA. Although we agree that this

histologic pattern is unique, we suggest that it may represent an unusual response to staphylococci, rather than a reaction pattern specific to MRSA.

As expected, significant differences in susceptibility patterns between MRSA and MSSA isolates were evident in the study reported here. Resistance to the fluoroquinolones marbofloxacin and enrofloxacin and to the macrolides erythromycin and clindamycin was complete among MRSA strains, whereas MSSA strains maintained a high frequency of susceptibility to these antimicrobials. Analysis of SCCmec typing results revealed that all isolates contained a type II SCCmec element, which is consistent with the broad antimicrobial resistance patterns of these organisms. Strains that carry SCCmec type II, which are generally associated with hospital-acquired MRSA infections in humans, coexpress determinants for resistance to non- β -lactam antimicrobials, such as fluoroquinolones and macrolides.²²

Strains that carry SCCmec type IV are most commonly associated with community-acquired-MRSA strains, which have maintained antimicrobial susceptibility patterns comparable to those for MSSA isolates (with the obvious exception of resistance to β -lactam antimicrobials).²² We had expected MRSA isolates obtained from cats to carry the SCCmec type IV element and assumed that cats would be more likely to contract strains that circulate commonly in the human community. Despite the fact that 14 of 15 strains were highly related on the basis of results of PFGE analysis, it is quite unlikely that the cats contracted their MRSA strains from a common veterinary nosocomial source because none of the cats were referred to our facility by the same veterinarian and most were first-time admissions into our hospital, with infections already active prior to admission.

To our knowledge, the study reported here is the first to reveal MRSA strains with an SCCmec type II element in domestic pets. Reverse-zoonotic transmission from humans to domestic animals must be assumed because SCCmec type II strains have evolved in the human healthcare setting. Epidemiologic studies to track and correlate human risk factors for the carriage of, or infection by, SCCmec type II MRSA strains with the carriage or infection of cats are needed to determine the relative risk that cats could pose for transmission to humans. Because MRSA infections of cats do not appear to differ in clinical signalment from MSSA infections of cats, diligence is necessary on the part of veterinarians to suspect MDR infections early during unsuccessful empirical antimicrobial treatment and to submit samples for culture and susceptibility testing in a timely manner.

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- d. Wizard genomic DNA purification kit, Epicentre Technologies, Madison, Wis.
- e. Techne gradient thermal cycler, Techne, Princeton, NJ.
- f. StatXact, version 6.1, Cytel Software Corp, Cambridge, Mass.

- g. SAS software, version 9.1, SAS Institute Inc, Cary, NC.
- h. Microbank, ProLab Diagnostics, Austin, Tex.

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