

Emergence of methicillin-resistant *Staphylococcus pseudintermedius* in Switzerland: Three cases of urinary tract infections in cats

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Summary

Methicillin resistance has emerged in clinical isolates of *Staphylococcus pseudintermedius* from cats in Switzerland. Three cats suffering from urinary tract infections were infected with methicillin-resistant *S. pseudintermedius* (MRSP). Phenotypic and genotypic characterization of the resistance profile showed that the isolates displayed resistance to all beta-lactams and cephalosporins (*blaZ*, *mecA*), fluoroquinolones, tetracyclines [*tet(K)*], macrolides, lincosamides and streptogramins B [*erm(B)*], chloramphenicol (*cat_{pC221}*), trimethoprim [*dfr(G)*] and the aminoglycosides gentamicin [*aac(6′)-Ie-aph(2′)-Ia*], kanamycin and neomycin [*aph(3′)-III*] and streptomycin [*ant(6)-Ia*]. They also harbor the leukocidin gene *lukS-I*. MRSP represents a new challenge for antibiotic therapy and this zoonotic bacteria may rapidly spread to animals and humans.

Keywords: *Staphylococcus pseudintermedius*, leukocidin, methicillin-resistance, cat, urinary tract infection

Methicillin-resistente *Staphylococcus pseudintermedius* in der Schweiz: Drei Fälle von Katzen mit Harnwegsinfektionen.

Methicillin-resistente *Staphylococcus pseudintermedius* (MRSP) sind ein neu auftretendes Problem in der Tiermedizin in der Schweiz. Wir beschreiben drei Fälle, in welchen Methicillin-resistente *Staphylococcus pseudintermedius* aus Urin von Katzen mit Harnwegsinfektionen isoliert wurden. Die phänotypische und genotypische Untersuchung des Resistenzprofils zeigte Resistenzen gegen Beta-Laktame und Cephalosporine (*blaZ*, *mecA*), Fluorochinolone, Tetrazykline [*tet(K)*], Makrolide, Lincosamide und Streptogramin B [*erm(B)*], Chloramphenicol (*cat_{pC221}*), Trimethoprim [*dfr(G)*], Gentamicin [*aac(6′)-Ie-aph(2′)-Ia*], Kanamycin und Neomycin [*aph(3′)-III*] sowie Streptomycin [*ant(6)-Ia*]. Bei allen drei Isolaten wurde das leukocidin Gen *lukS-I* nachgewiesen. MRSP haben ein zoonotisches Potential und stellen somit eine Gefahr für die Gesundheit von Menschen und Tieren dar.

Schlüsselwörter: *Staphylococcus pseudintermedius*, Leukocidin, Methicillin-Resistenz, Katze, Harnwegsinfektion.

Introduction

This past year, methicillin-resistant *S. pseudintermedius* have emerged in clinical isolates from dogs and cats in Switzerland and have been isolated with increasing frequency at the diagnostic unit of the Institute of Veterinary Bacteriology of the University of Berne. Methicillin resistance in *Staphylococcus* is determined by resistance to oxacillin and by the presence of the *mecA* gene which confers resistance to all β -lactam and cephalosporin antibiotics (Deurenberg et al., 2007). Methicillin-resistant

Staphylococcus are often resistant to other commonly used antibiotics.

S. pseudintermedius is a novel species that was described in 2005 by Devriese et al. (Devriese et al., 2005). Since its discovery, *S. pseudintermedius* has mainly been isolated from dogs (Hanselman et al., 2008) and was also found in the nasal cavities of people working with dogs (Sasaki et al., 2007a). It can also cause infections in humans (Van Hoovels et al., 2006). Two recent studies have shown that *S. pseudintermedius*, not *S. intermedius*, is responsible for infections in dogs and cats (Bannoehr et al., 2007;

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Sasaki et al., 2007b). In routine laboratory diagnostics, *S. pseudintermedius* can be easily misidentified as *S. aureus* and is not distinguishable from *S. intermedius* using conventional biochemical methods. They share the same phenotypic features as β -hemolysis on blood agar plates, coagulase and DNase production. *S. pseudintermedius* and *S. intermedius* can be differentiated from *S. aureus* on chromagar Uri select 4 (Bio-Rad, Hercules, CA) where colonies display different colors, or by using API galleries (bioMérieux, Marcy l'Etoile, France). However, molecular methods are necessary to differentiate *S. pseudintermedius* from *S. intermedius* (Van Hoovels et al., 2006; Sasaki et al., 2007a). Recently, methicillin-resistant *S. pseudintermedius* (MRSP) have emerged in dogs, which act as a reservoir for such an opportunistic pathogen (Descloux, 2007; Sasaki et al., 2007a; Hanselman et al., 2008). A fifth of the *S. pseudintermedius* isolated from the ears and nasal cavities of dogs in Switzerland were also found to carry the leukocidin gene *lukS-I* which causes tissue necrosis (Prévost et al., 1995; Descloux, 2007).

Animals, material and methods

Case 1: *S. pseudintermedius* KMA1450

A castrated male Chartreux cat that was born in 1997 was presented for the first time to the veterinarian in 1999. It was suffering from FLUTD (Feline lower urinary tract disease) with hemorrhagic cystitis. Urine sediment was examined microscopically and no concrements were found. The cat was given amoxicillin. After therapy, hematuria returned and the cat was treated again with one injection of ampicillin and put on a low pH diet. During the next 6 years, the cat was treated twice with antibiotics (clindamycin and cefalexin) for other lesions not associated with urinary tract infection. In 2006, the cat again suffered from FLUTD. The veterinarian referred the cat to a veterinary hospital. The cat received a wet feline urinary tract diet and enrofloxacin. One month later, the cat still showed stranguria and *S. pseudintermedius* (strain KMA1450) together with *Enterococcus faecalis* were detected in urine culture. A perineal urethrostomy was made and the cat was treated with marbofloxacin and feline urinary tract diet was continued. The cat recovered and had no further urinary problems.

Case 2: *S. pseudintermedius* KMA690

In May 2007, a 5 year old castrated male European cat was referred by the veterinarian to a veterinary hospital for perineal urethrostomy. The cat had demonstrated urination problems for two years. It was pre-treated several times with antibiotics (details of substances could not be made available). In the hospital, the cat had signs of polakiuria and stranguria. It was treated with a combination of amoxicillin-clavulanic acid and analgesics, but with

no success. As a result, perineal urethrostomy was performed. Bacteriological analyses of urine sample revealed the presence of multidrug-resistant *S. pseudintermedius* (strain KMA690). After surgery the cat returned home, but urinary problems persisted, requiring treatment twice with an antibiotic regimen.

Case 3: *S. pseudintermedius* IMD720

In June 2007, a 9 year old castrated male European cat was presented to the veterinarian with FLUTD and urethral obstruction. Ultrasonography was performed and cystitis with concrements and suspicion of bilateral nephritis was diagnosed. The cat was treated with analgesics and marbofloxacin combined with a feline urinary tract diet. After a week at home, the cat again suffered from stranguria. Urine analysis revealed the presence of triple phosphates and *S. pseudintermedius* (strain IMD720). The veterinarian changed antibiotic therapy and prescribed the combination amoxicillin-clavulanic acid. One week later, the cat was inappetent, started vomiting and suffered from dysuria and stranguria. The veterinarian and the owner decided to euthanize the cat.

Isolation and identification of *S. pseudintermedius*

Urinary samples were taken by cystocentesis (case 2 and 3) or by voiding the bladder by manual pressure (case 1). Urine specimens were analyzed immediately or within two days. For bacterial culture, 10 μ l of urine were spread on tryptone soy agar (TSA) agar plates containing 5% sheep blood (Oxoid Ltd., Basingstoke, England). The plates were incubated at 37° C in aerobic condition. After 24 hours, hemolysis pattern was observed and catalase activity was tested. The cells were Gram-stained and examined under light microscope. Isolates were identified using either the API ID32 STAPH or the RapidID32 Strept galleries (bioMérieux, Marcy l'Etoile, France). *S. pseudintermedius* isolates were identified by sequencing species-specific 16S rDNA and *sodA* gene, as described previously (Poyart et al., 2001; Kuhnert et al., 2002; Sasaki et al., 2007a). The leukocidin gene *lukS-I* was detected by PCR as described previously (Descloux 2007). Clonality was determined by multilocus sequence typing (Bannoehr et al., 2007)

Antimicrobial susceptibility testing and detection of antibiotic resistance genes. Minimal inhibitory concentrations (MICs) were determined in Mueller-Hinton broth by use of custom Sensititre susceptibility plates NLV57 (Trek Diagnostics Systems, East Grinstead, England, and MCS Diagnostics, BV, Swalmen, The Netherlands) according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2006a). Antibiotic resistance genes and the leukocidin gene *lukS-I* were detected using a microarray (Fig. 1) capable of detecting 111 antibiotic resistance genes and 4 virulence genes known to be present in Gram-positive bacteria (Perreten et al., 2005). Genomic DNA for PCR and

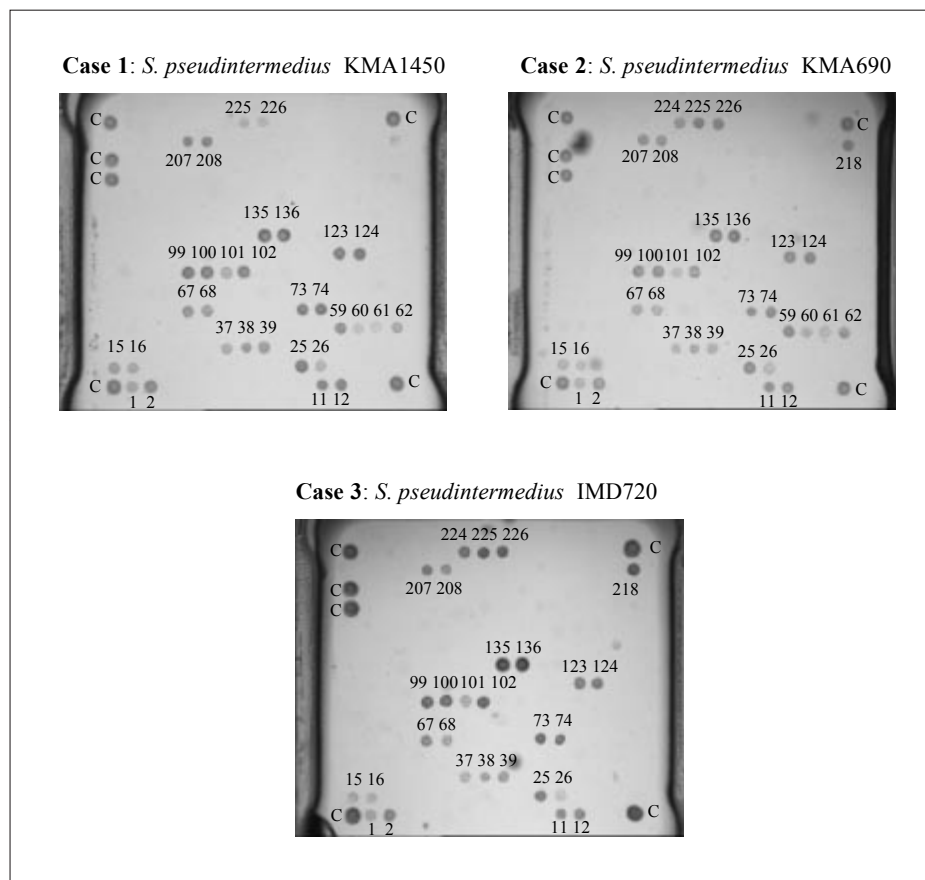


Figure 1. Microphotographs of microarrays hybridized with genomic DNA of *Staphylococcus pseudintermedius* KM1450, KM690 and IMD720. 1, 2: *aac(6′)-Ie*; 11, 12: *ant(6′)-a*; 15, 16: *aph(2′′)-Ia*; 25, 26: *aph(3′)-III*; 37, 38, 39: *bla_Z*; 59, 60, 61, 62: *cat_{pC221}*; 67, 68: *dfr(G)*; 73, 74: *erm(B)*; 99, 100, 101, 102: *mecA*; 123, 124: *sat4*; 135, 136: *tet(K)*; 207, 208: *lukS-I*; 218, 224, 225, 226: positive controls based on consensus of 16S rDNA sequences. C, biotin position marker. The custom Array-Tubes were manufactured by Clontech Chips Technologies, Jena, Germany.

microarray analyses was isolated using the E.Z.N.A. Bacterial DNA Kit (Peqlab Biotechnologie GmbH, Germany).

Results

Three of four isolates that grew on TSA were Gram-positive, catalase-positive, non-pigmented cocci and displayed double hemolysis (α - and β -hemolysis). They were identified as *S. intermedius* with API ID32 STAPH Galleries (bioMérieux, Marcy l’Etoile, France). Sequencing of species-specific 16S rDNA and *sodA* gene allowed for precise identification of the isolates as *S. pseudintermedius*. In all three cases, *S. pseudintermedius* was present in urine samples at a high concentration ($>10^4$ cfu/ml in case 1, 3×10^4 cfu/ml in case 2 and $>10^5$ cfu/ml in case 3). In case 1, the urine sample also contained *Enterococcus faecalis* (10^2 cfu/ml). This strain displayed resistance to tetracycline and was susceptible to other tested antibiotics.

The three *S. pseudintermedius* isolates were resistant to all classes of drugs allowed in veterinary medicine in Switzerland. They were still susceptible to antibiotics of major importance in human medicine such as linezolid, nitrofurantoin, quinupristin-dalfopristin and vancomycin. The MICs of 18 antibiotics for the three *S. pseudintermedius* isolates and the detected antibiotic resistance genes are

presented in Table 1 and Figure 1. They all belong to the same clonal lineage ST 71 and contained the necrotizing leukocidin LukS-I. Out of the three cats infected with *S. pseudintermedius*, one had to be euthanized (case 3) and two underwent surgery consisting of perineal urethrostomy (case 1 and 2). Surgery allowed cat 1 to fully recover while cat 2 still suffers from occasional urinary problems and has already been twice under antibiotic treatment.

Discussion

This is the first description of cases of infections in cats caused by MRSP carrying a leukocidin gene in Switzerland. The use of β -lactam antibiotics or fluoroquinolones may have contributed to selection for MRSP in animals since the use of these drugs has been shown to represent a risk factor for the acquisition of methicillin-resistant *S. aureus* in humans (15). In our three cases, all three cats received antibiotics belonging to these classes of drugs before the MRSP were isolated. Furthermore, the presence of multidrug-resistance in a bacterium that is specific to animals demonstrates that resistances have been selected for in animals rather than in humans. MRSP carrying a cytotoxin represent new therapeutic challenges. One of the three cats infected with *S. pseud-*

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Table 1: Minimal inhibitory concentrations (MICs) of 18 antibiotics for *Staphylococcus pseudintermedius* strains isolated from urinary tract infections of cats and antibiotic resistance genes.

Antibiotics	<i>S. pseudintermedius</i>					
	KMA1450 MIC (µg/ml)	Genes	KMA690 MIC (µg/ml)	Genes	IMD720 MIC (µg/ml)	Genes
Amikacin	≤ 16		≤ 16		≤ 16	
Amoxicillin/clavulanic acid ³⁾	16/8	<i>mecA</i>	32/16	<i>mecA</i>	16/8	<i>mecA</i>
Cephalothin	>32	<i>mecA</i>	>32	<i>mecA</i>	16	<i>mecA</i>
Chloramphenicol	64	<i>cat_PC221</i>	64	<i>cat_PC221</i>	8	
Clindamycin	>8	<i>erm(B)</i>	>8	<i>erm(B)</i>	>8	<i>erm(B)</i>
Enrofloxacin	>16		>16		>16	
Erythromycin	>16	<i>erm(B)</i>	>16	<i>erm(B)</i>	>16	<i>erm(B)</i>
Gentamicin	500	<i>aac(6′)-Ie-aph(2′)-Ia</i>	32	<i>aac(6′)-Ie-aph(2′)-Ia</i>	500	<i>aac(6′)-Ie-aph(2′)-Ia</i>
Kanamycin	>1000	<i>aph(3′)-III</i>	512	<i>aph(3′)-III</i>	>1000	<i>aph(3′)-III</i>
Linezolid	≤ 0.5		1		1	
Nitrofurantoin	≤ 16		≤ 16		≤ 16	
Oxacillin	>16	<i>mecA</i>	>16	<i>mecA</i>	>16	<i>mecA</i>
Penicillin	>8	<i>bla_Z, mecA</i>	>8	<i>bla_Z, mecA</i>	>8	<i>bla_Z, mecA</i>
Streptomycin	>32	<i>ant(6)-Ia</i>	>32	<i>ant(6)-Ia</i>	>32	<i>ant(6)-Ia</i>
Streptothricin	nd	<i>sat4</i>	nd	<i>sat4</i>	nd	<i>sat4</i>
Trimethoprim/sufamethoxazole a)	8/152	<i>df_r(D)</i>	8/152	<i>df_r(D)</i>	8/152	<i>df_r(D)</i>
Quinupristin-dalfopristin	1		1		1	
Tetracycline	>32	<i>tet(K)</i>	>32	<i>tet(K)</i>	>32	<i>tet(K)</i>
Vancomycin	≤ 1		≤ 1		≤ 1	

intermedius had to be euthanized while the other two underwent surgery consisting of perineal urethrostomy. This report shows the importance of an accurate bacteriological diagnostic and interpretation of antibiograms according to guidelines. Oxacillin or ceftiofur should be used to predict *mecA*-mediated resistance in staphylococci since methicillin-resistant staphylococci may falsely appear susceptible to penicillins and cephalosporins *in vitro* (Clinical and Laboratory Standards Institute, 2006b). This could have helped avoiding random and inactive antimicrobial therapy and selection for resistant bacteria, as well as surgery. Before surgery, alternative therapies such as the use of a therapeutic diet in combination with an appropriate antibiotic have to be considered. The emergence of zoonotic MRSP infections represents a threat for human and animal health. MRSP may rapidly

spread through the animal and human population as is happening with MRSA (van Duijkeren et al., 2004; Weese et al., 2005; Abraham et al., 2007). Appropriate use of antibiotics as well as guidelines for antibiotic use in veterinary medicine are necessary to limit the selection for and spread of such a multidrug-resistant and pathogenic bacteria to the community and hospitals.

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Apparition de *Staphylococcus pseudintermedius* résistants à la méthicilline en Suisse: 3 cas d'infections urinaires chez le chat

Les *Staphylococcus pseudintermedius* résistants à la méthicilline (MRSP) sont un problème nouveau en médecine vétérinaire en Suisse. Nous décrivons 3 cas dans les quels des *Staphylococcus pseudintermedius* résistants à la méthicilline ont été isolés dans l'urine de chats souffrant des voies urinaires. L'examen phénotypique et génotypique du profil de résistance a montré des résistances contre les beta-lactame et les céphalosporine (*bla_Z, mecA*) les fluorochinolones, les

Emergenza in Svizzera per lo *Staphylococcus pseudintermedius* resistente alla meticillina. Infezione del tratto urinario in tre gatti

Lo *S. pseudintermedius* (MRSP), resistente alla meticillina, rappresenta un problema nascente nella medicina veterinaria in Svizzera. Qui descriviamo tre casi in cui si è isolato lo *S. pseudintermedius* resistente alla meticillina nell'urina di tre gatti con infezione urinaria. L'analisi del genotipo e del fenotipo dei profili di resistenza hanno mostrato una resistenza contro: betalactame e cefalosporine (*bla_Z, mecA*), fluorochinolone, tetracicline [*tet(K)*], macrolidi, lincosamidi e

tétracyclines, [*tet*(K)], les macrolides, les lincosamides et streptogramines B [*erm*(B)], le chloramphénicol (*cat*_PC221), le triméthoprime, [*dfr*(G)] la gentamicine [*aac*(6')-*le-aph*(2')-*la*], la kanmycine et la néomycine [*aph*(3 primes) III] ainsi que la streptomycine [*ant*(6)-*la*]. Dans les 3 isolats le gène leucocidine (*lukS-I*) a été mis en évidence. Les MRSP ont un potentiel de zoonose et représentent donc un danger pour la santé humaine et animale

streptogramina B [*erm*(B)], cloroamfenicolo (*cat*_PC221), trimetropin [*dfr*(G)], gentamicina [*aac*(6')-*le-aph*(2')-*la*], canamicina e neomicina [*aph*(3')-III] e infine streptomycina [*ant*(6)-*la*]. In tutti e 3 i casi isolati si è rilevata leucocidina Gen *lukS-I*. Gli MRSP hanno un potenziale zoonotico e sono un pericolo per la salute degli uomini e degli animali.

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