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Original article

Antibiotic resistance of canine *Staphylococcus intermedius* group (SIG) – practical implications

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Abstract

A total of 221 SIG strains were isolated from clinical samples of canine origin submitted to the Diagnostic Laboratory of the Division of Bacteriology and Molecular Biology at the Warsaw University of Life Sciences in Warsaw during the period 2006-2010. The aim of the study was to investigate the frequency of prevalence of methicillin-resistant SIG strains and to determine the MIC values of cephalotin, amoxicillin/clavulanic acid, ciprofloxacin, clindamycin, gentamicin, chloramphenicol, mupirocin for a collection of randomly selected 79 strains belonging to *Staphylococcus intermedius* group (SIG), including 23 *mecA*-positive and 56 *mecA*-negative strains. All isolates were identified as belonging to SIG based on their phenotypic properties and PCR amplification of *S. intermedius*-specific fragment of the 16S rRNA gene. The *mecA* gene was detected in 26 (12%) of 221 SIG strains. All tested *mecA*-negative SIG strains were susceptible to amoxicillin/clavulanic acid and cephalotin. One of the 56 *mecA*-negative SIG strains was resistant to ciprofloxacin, six (11%) to gentamicin. It was found that sixteen (29%) of 56 *mecA*-negative SIG strains were resistant to clindamycin. Most of the *mecA*-positive SIG strains were resistant to ciprofloxacin (96%), clindamycin (96%), and gentamicin (96%). Only one MRSIG strain was resistant to chloramphenicol. All examined *mecA*-positive SIG strains were found to be susceptible to mupirocin. Our results imply that staphylococcal multidrug resistance has become more prevalent, which could lead to difficulties in effective treatment. With some resistant strains the only therapeutic possibility are antimicrobial agents important in human medicine. New regulations for veterinary medicine concerning appropriate therapy of infections caused by multidrug-resistat staphylococci are needed.

Key words: *Staphylococcus intermedius* group, MIC, *mecA* gene, antibiotic resistance, dogs

Introduction

Staphylococcus intermedius was first described as a new coagulase-positive species in 1976 (Hajek 1976). It is a member of the normal flora of dogs and also a major opportunistic pathogen responsible for common canine skin infections. *S. intermedius* has also been found in a wide range of other animals and can occasionally cause severe infections in humans (Mahoudeau et al. 1997, Tanner et al. 2000, Bes et al. 2002, Futagawa-Saito et al. 2004). During the past few years, there has been confusion about the identifica-

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tion of this species. Using a multilocus sequencing approach, independent research groups have demonstrated that isolates phenotypically identified as *Staphylococcus intermedius* consist of three distinct species, including *S. intermedius*, *S. pseudintermedius*, and *S. delphini*, which together represent the *S. intermedius* group (SIG) (Bannoehr et al. 2007, Devriese et al. 2008, Bannoehr et al. 2009). Importantly, it was discovered that *S. pseudintermedius*, but not *S. intermedius*, is the common etiological agent of canine pyoderma and that *S. delphini*, isolated from a variety of different animals, may be more clinically important than was previously thought (Bannoehr et al. 2007, Devriese et al. 2008, Bannoehr et al. 2009). However, there is no "gold standard" to differentiate phenotypically among SIG strains.

The most common causes of antimicrobial treatment in dogs are skin and wound infections, otitis externa, respiratory and urinary tract infections. Some canine infections (e.g. deep skin pyoderma and some forms of otitis externa) often require repeated and prolonged antimicrobial treatment. Difficult cases are often treated with fluoroquinolones and can involve continuous therapy for periods as long as 7 months (Carlotti et al. 1999, Guardabassi et al. 2004). There is some opinion that resistance patterns of SIG are quite predictable and in contrast to well known multidrug-resistant MRSA (methicillin-resistant *Staphylococcus aureus*), staphylococci from SIG isolated from animals are regarded as sensitive to antimicrobials. Results of antibiotic resistance testing are significant, because of the rising incidence of isolation of methicillin-resistant SIG (MRSIG). In the past, these strains were reported to be susceptible to beta-lactam antibiotics, but methicillin-resistant SIG, particulary *S. pseudintermedius* (MRSP) strains are being reported with increasing frequency (Gortel et al. 1999, Vengust et al. 2006, Zubeir et al. 2007, van Duijkeren et al. 2008). Methicillin-resistant *S. pseudintermedius* have been isolated from dogs, cats and humans (Hanselman et al. 2007, Sasaki et al. 2007, Wettstein et al. 2008). As in MRSA, the methicillin resistance of *S. pseudintermedius* is mediated by the *mecA* gene. Strains isolated from clinical samples, especially with history of previous antibiotic treatment are very often multiresistant. It is well documented for many organisms, including SIG strains, that the clinical use of antimicrobial drugs selects for resistant bacteria (Prescott et al. 2002, Guardabassi et al. 2004, Loeffler et al. 2007). In 2006, a sudden rise in isolation of methicillin-resistant SIG (MRSIG) from clinical specimens of animal origin was noted (Ruscher et al. 2009).

There have been many studies on the *in vitro* effect of antimicrobial agents against SIG strains isolated from dogs in countries outside Poland. However, few studies of the antimicrobial susceptibility patterns of these isolates from dogs in Poland have been reported (Hauschild and Wójcik 2007, Kizerwetter-Świda et al. 2009).

The present study was designed to asses the prevalance of methicillin resistance among SIG strains of canine origin and to determine the MIC values of cephalotin, amoxicillin/clavulanic acid, ciprofloxacin, clindamycin, gentamicin, chloramphenicol and mupirocin for a collection of randomly selected 79 SIG strains, including MRSIG, isolated from dogs.

Materials and Methods

Isolation and identification of SIG strains. A total of 221 SIG strains were isolated from the canine clinical samples submitted to the Diagnostic Laboratory of the Division of Bacteriology and Molecular Biology at the Warsaw University of Life Sciences in Warsaw during the period 2006-2010. Minimum inhibitory concentrations (MICs) of selected antimicrobials which are quantitatively most used in animals were determined for a subset of randomly selected 79 SIG strains (Table 1). The bacteria were cultured on Columbia agar supplemented with 5% sheep blood (bioMerieux) in aerobic conditions. Identification of isolated strains was based on their phenotypic characteristics, using standard bacteriological procedures described by Malicki and Binek (2004). The following tests were used: colony pigment, type of hemolysis, coagulase, clumping factor and acid production from maltose. Biochemical activities of the isolates were examined using API Staph test (bioMerieux). Additionally, the PCR assay based on the amplification of 16S rRNA gene of *S. intermedius* was used for confirmation of identification of SIG strains (Wakita et al. 2002). *Staphylococcus intermedius* ATCC 29663 and methicillin-resistant *Staphylococcus aureus* MIKROBANK 14.002 were used as the control form PCR and determination of MICs.

Table 1. Origin of 79 randomly selected SIG isolates used in the study.

Clinical sample	Number of isolates	Total number of isolates used in the study
External auditory canal swab	27	79
Skin swab	26	
Urine	9	
Conjunctival swab	5	
Nasal cavity swab	4	
Pharyngeal swab	3	
Vaginal swab	2	
Pseudojoint swab	1	
Prostatic gland swab	1	
Femoral canal swab	1	

Table 2. Breakpoints of studied antimicrobials used for evaluation of MIC values.

Antimicrobial agent	Breakpoint values	Recommendations	Comments
Amoxicillin/clavulanic acid (XL)	≥ 8	CLSI	Human data
Cephalotin (CE)	≥ 32	CLSI	Human data
Clindamycin (CM)	≥ 4	CLSI	Veterinary data
Gentamicin (GM)	≥ 8	CLSI	Human data
Ciprofloxacin (CI)	> 1	EUCAST	Human data
Chloramphenicol (CL)	> 8	EUCAST	Human data
Mupirocin (MU)	≥ 4	*	

* The CLSI/EUCAST have no interpretive criteria for mupirocin; the mupirocin interpretive criteria were based on published reports (Finlay et al. 1997, Oliveira et al. 2007).

Detection of *mecA* gene. All 221 strains were tested for the presence of *mecA* by PCR. DNA for amplification was extracted from single colonies with Genomic Mini kits (A&A Biotechnology) using lisostaphin (100 µg/ml, Sigma). A pair of primers mecA1 5'-AAAATCGATGGTAAAGGTTGGC-3' and mecA2 5'-AGTTCTGCAGTACCGGATTTGC-3', described by Strommenger et al. (2003) were used to amplify a *mecA* gene fragment of 532 bp in size. Amplification reactions were performed in a volume of 50 µl, all reagents were purchased from Fermentas. The presence of PCR products was analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide using a VersaDoc Model 1000 Imaging System with Quantity One 4.4.0 software (BioRad).

Determination of MICs. E-test® strips (bioMerieux) were used for determining the minimum inhibitory concentration (MIC) of ciprofloxacin (CI), clindamycin (CM), gentamicin (GM) for all 79 strains, and additionally cephalotin (CE), amoxicillin/clavulanic acid (XL) for 56 *mecA*-negative SIG strains, and chloramphenicol (CL), mupirocin (MU) for 23 *mecA*-positive SIG strains. Breakpoints of studied antimicrobial agents provided by the CLSI and EUCAST were used for evaluation of MIC values (Table 2).

Results

All 221 isolates were identified as SIG strains based on their phenotypic properties and PCR amplification of the *S. intermedius*-specific fragment of the 16S rRNA gene. As it has been shown that 16S rRNA gene sequences from the three SIG species are 99% identical, our isolates could be characterized only as belonging to the SIG. Detailed identification to the species level requires additional molecular biology based techniques, which are in progress. Analysis of biochemical properties by numerical API Staph codes revealed in some cases identification of *Staphylococcus intermedius*. But some codes were not included in product database, thus some strains were not identified to the species level. The *mecA* gene was identified in 26 (12%) out of 221 SIG strains. MIC values of selected antimicrobials were determined for a subset of randomly selected 79 SIG strains – 56 *mecA*-negative, and 23 *mecA*-positive. Table 3 shows that all 56 *mecA*-negative SIG strains (MSSIG) were susceptible to amoxicillin/clavulanic acid and cephalotin. One of the 56 *mecA*-negative SIG strains was resistant to ciprofloxacin, six (11%) to gentamicin. It was found that sixteen (29%) of 56 *mecA*-negative SIG strains were resistant to clindamycin (Table 3).

Most of the *mecA*-positive SIG strains were resistant to ciprofloxacin (96%), clindamycin (96%), and gentamicin (96%). Only one MRSIG strain was resistant to chloramphenicol. All examined *mecA*-positive SIG strains were found to be susceptible to mupirocin (Table 4).

Discussion

In recent years, antimicrobial resistance in bacteria of animal origin, including food-producing and household pets has gained particular attention. Staphylococci isolated from animals are regarded as relatively sensitive to antimicrobial agents. However, the latest findings from antimicrobial susceptibility testing revealed a remarkable increase in prevalance of methicillin-resistant staphylococci isolated from animals (Malayeri et al. 2010). Occurence of *mecA* genes in canine staphylococci varies in different countries. We have identified 12% of examined strains as methicillin-resistant based on the presence of the *mecA* gene. In our study, canine SIG strains were divided into two groups (*mecA*-negative and *mecA*-positive). *MecA*-negative isolates were sensitive to amoxicillin with clavulanic acid and cephalotin. There were low resistance rates to ciprofloxacin and gentamicin among these strains and moderate resistance to clindamycin. Our results concerning *mecA*-negative SIG isolates are in line with findings from the literature, but it must be remembered that they were compared to previously reported resistance

Table 3. MIC values of studied antimicrobial agents for MSSIG (n=56) strains.

Antimicrobial agent	Susceptible MIC range (μg/ml) Number of strains / %	Resistant MIC range (μg/ml) Number of strains / %	MIC^{9}_{0} (μg/ml)
Amoxicillin/clavulanic acid (XL)	0.047 – 0.19 56/100%	–	0.19
Cephalotin (CE)	0.047 – 0.125 56/100%	–	0.125
Clindamycin (CM)	0.064 – 1.0 40/71%	32 1/2% >256 15/27%	>256
Ciprofloxacin (CI)	0.047 – 0.5 55/98%	>32 1/2%	0.125
Gentamicin (GM)	0.19 – 0.75 50/90%	24 – 96 6/10%	24

Table 4. MIC values of studied antimicrobial agents for MRSIG (n=23) strains.

Antimicrobial agent	Susceptible MIC range (μg/ml) Number of strains / %	Resistant MIC range (μg/ml) Number of strains / %	MIC^{9}_{0} (μg/ml)
Chloramphenicol (CL)	3.0 – 6.0 22/96%	32 1/4%	6.0
Mupirocin (MU)	0.064 – 0.19 23/100%	–	0.125
Clindamycin (CM)	0.38 1/4%	>256 22/96%	>256
Ciprofloxacin (CI)	0.25 1/4%	>32 22/96%	>32
Gentamicin (GM)	0.38/1 1/4%	16 – 384 22/96%	192

patterns of *S. intermedius*. We found that 29% of strains were resistant to clindamycin. Similar resistance levels of *S. intermedius* to this drug were observed by other authors, 39% (Lyskova et al. 2007), 22% (Hartmann et al. 2005), 15% (Oliveira et al. 2008) and 9.64% (Vanni et al. 2009). In contrast, findings from 1995 demonstrated very effective activity of clindamycin against staphylococci (Dowling 1995). Interestingly, a similar study of *S. intermedius* isolated in Poland revealed that 31.57% of them were resistant to clindamycin (Hauschild and Wójcik 2007). None of these strains was resistant to gentamicin in contrast to our strains, 10% of which showed resistance to this antibiotic. This may be correlated with the excessive topical use of veterinary products containing gentamicin. In general, *S. intermedius* isolated from dogs are characterized by some authors to be fully susceptible to gentamicin (Hartmann et al. 2005, Lyskova et al. 2007) and ciprofloxacin. However, some strains might be resistant according to other authors in 3.03% (Malayeri et al. 2010) and 1.75% (Vanni et al. 2009). Effective activity of amoxicillin with clavulanic acid and cephalotin against these strains was observed. On the other hand, there is high resistance level to penicillin G and tetracycline (Hartmann et al. 2005, Lyskova et al. 2007, Vanni et al. 2009, Malayeri et al. 2010).

It is well known that increasing resistance to antimicrobials is associated with the extensive use of these drugs in veterinary practice. This may be an explanation for the increase in the prevalance of methicillin-resistant staphylococci isolated from animals. As expected, resistance is especially high among these strains, which is correlated with the characteristics of the *mecA* gene and staphylococcal cassette chromosome *mec* (SCC*mec*). *MecA*-positive SIG strains of animal origin are multidrug-resistant, similarly to MRSA. They are resistant to β-lactams, aminoglycosides, macrolides and fluoroquinolones. Our results confirm multidrug resistance of *mecA*-positive SIG strains. We have found high resistance levels to clindamycin, ciprofloxacin and gentamicin. Only one of

23 of the examined strains was resistant to chloramphenicol and all were susceptible to mupirocin. This is in agreement with observations of other authors (Loeffler et al. 2007, Sasaki et al. 2007, Epstain et al. 2009, Ruscher et al. 2009).

Antimicrobials used in veterinary dermatology include: amoxicillin with clavulanic acid, cephalosporins, fluoroquinolones and lincosamides. Empirical therapy may be succesful in infections caused by methicillin-sensitive staphylococci. However, in the case of multidrug-resistant *mecA*-positive strains practitioners cannot obtain a full resolution in empirical therapy. Thus, bacterial culture and antimicrobial susceptibility testing is strongly recommended, especially in refractory cases.

In general methicillin-resistant staphylococci of canine origin are sensitive to mupirocin, rifampicin, linezolid and vancomycin, which are important in human medicine for treatment of MRSA infections or represent "antimicrobial agents of last resort" (Perreten et al. 2010). Treatment of any infections caused by *mecA*-positive SIG strains may be a challenge. Antimicrobial therapy must be based on the result of reliable resistance testing. There is only a limited number of drugs which may be used. One of the effective antimicrobials active against Gram-positive bacteria, including methicillin-resistant stahylococci, is mupirocin, occasionally used topically in veterinary medicine to treat canine pyoderma. Mupirocin is available as a veterinary ointment in the US, and can be prescribed at any pharmacy in other countries. Fulham et al. (2010) described a high level of *in vitro* sensitivity of methicillin-resistant staphylococci of canine origin to mupirocin. As far as the authors are aware, the first case of infection after joint prothesis implantation in a dog caused by MRSP in Poland involves only one strain. This strain showed *in vitro* susceptibility to mupirocin, linezolid, chloramphenicol and vancomycin. Infection was successfully treated using linezolid (Międzobrodzki et al. 2010).

Based on the results of our study it can be stated that the majority of canine infections caused by staphylococci may be succesfully treated using amoxicillin with clavulanic acid or cephalotin. However, the increase in the occurrence of *mecA*-positive strains belonging to SIG is significant. In these cases empirical therapy will not be effective, and therefore antibiotic sensitivity testing is recommended. Veterinary personnel and pet owners may become colonized by MRSP, and therefore staff working in veterinary clinics should be made aware of the risk of nosocomial transmission of MRSP. New guidelines concerning antimicrobial therapy of multidrug-resistant staphylococci in veterinary medicine should be produced. Antimicrobial agents should be used only when necessary, after antimicrobial sensitivity testing, with correct dosage and administration. Prophylactic use of antimicrobials should be avoided. Moreover, there is a need for rigorous hygiene protocols to prevent transfer of MRSP in veterinary settings.

References

Bannoehr J, Ben Zakour NL, Waller AS, Guardabassi L, Thoday KL, van den Broek AH, Fitzgerald JR (**2007**) Population genetic structure of the *Staphylococcus intermedius* group: insights into *agr* diversification and the emergence of methicillin-resistant strains. J Bacteriol 189: 8685-8692.

Bannoehr J, Franco A, Iurescia M, Battisti A, Fitzgerald JR (**2009**) Molecular diagnostic identification of *Staphylococcus pseudintermedius*. J Clin Microbiol 47: 469-471.

Bes M, Saidi SL, Becharnia F, Meugnier H, Vandenesch F, Etienne J, Freney J (**2002**) Population diversity of *Staphylococcus intermedius* isolates from various host species: typing by 16S-23S intergenic ribosomal DNA spacer polymorphism analysis. J Clin Microbiol 40: 2275--2277.

Carlotti DN, Guaguere E, Pin D, Jasmin P, Thomas E, Guiral V (**1999**) Therapy of difficult cases of canine pyoderma with marbofloxacin: a report of 39 dogs. J Small Anim Pract 40: 265-270.

Clinical Laboratory Standards Institute (CLSI) (**2008**) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard – Third edition. M31-A3, Vol.28, No.8. CLSI, Wayne PA.

Devriese LA, Hermans K, Baele M, Haesebrouck F (**2008**) *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius.* Vet Microbiol 133: 206-207.

Dowling PM (**1996**) Antimicrobial therapy of skin and ear infections. Can Vet J 37: 695-699.

Epstein CR, Yam WC, Peiris JS, Epstein RJ (**2009**) Methicillin-resistant commensal staphylococci in healthy dogs as a potential zoonotic reservoir for community-acquired antibiotic resistance. Infect Genet Evol 9: 283--285.

Finlay JE, Miller LA, Poupard JA (**1997**) Interpretive criteria for testing susceptibility of staphylococci to mupirocin. Antimicrob Agents Chemother 41: 1137-1139.

Fulham KS, Lamarie SL, Hosgood G, Dick HL (**2011**) *In vitro* susceptibility testing of meticillin-resistant and meticillin-susceptible staphylococci to mupirocin and novobiocin. Vet Dermatol 22: 88-94.

Futagawa-Saito K, Suzuki M, Ohsawa M, Ohshima S, Sakurai N, Ba-Thein W, Fukuyasu T (**2004**) Identification and prevalence of an enterotoxin-related gene, se-int, in *Staphylococcus intermedius* isolates from dogs and pigeons. J Appl Microbiol 96: 1361-1366.

Gortel K, Campbell KL, Kakoma I, Whittem T, Schaeffer DJ, Weisiger RM (**1999**) Methicillin resistance among staphylococci isolated from dogs. Am J Vet Res 60: 1526-1530.

Guardabassi L, Schwarz S, Lloyd DH (**2004**) Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother 54: 321-332.

Hájek V (**1976**) *Staphylococcus intermedius*, a new species isolated from animals. Int J Syst Bacteriol 26: 401-408.

Hanselman BA, Kruth S, Weese JS (**2007**) Methicillin-resistant staphylococcal colonization in dogs entering a veterinary teaching hospital. Vet Microbiol 126: 277--281.

Hartmann FA, White DG, West SE, Walker RD, Deboer DJ (**2005**) Molecular characterization of *Staphylococcus intermedius* carriage by healthy dogs and comparison of antimicrobial susceptibility patterns to isolates from dogs with pyoderma. Vet Microbiol 108: 119-131.

Hauschild T, Wójcik A (**2007**) Species distribution and properties of staphylococci from canine dermatitis. Res Vet Sci 82: 1-6.

Kizerwetter-Świda M, Chrobak D, Rzewuska M, Binek M (**2009**) Antibiotic resistance patterns and occurence of *mecA* gene in *Staphylococcus intermedius* strains of canine origin. Pol J Vet Sci 12: 9-13.

Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JM, Winkler M, Weiss R, Lloyd DH (**2007**) First report of multiresistant, *mecA*-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Vet Dermatol 18: 412-421.

Lyskova P, Vydrzalova M, Mazurova J (**2007**) Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. J Vet Med A Physiol Pathol Clin Med 54: 559-563.

Mahoudeau I, Delabranche X, Prevost G, Monteil H, Piemont Y (**1997**) Frequency of isolation of *Staphylococcus intermedius* from humans. J Clin Microbiol 35: 2153--2154.

Malayeri HZ, Jamshidi S, Salehi TZ (**2010**) Identification and antimicrobial susceptibility patterns of bacteria causing otitis externa in dogs. Vet Res Commun 34: 435-444.

Malicki K, Binek M (**2004**) Outline of Clinical Veterinary Bacteriology (in Polish). 1st ed., Warsaw University of Life Sciences (SGGW) Press, Warsaw.

Międzobrodzki J, Kasprowicz A, Białecka A, Jaworska O, Polakowska K, Władyka B, Dublin A (**2010**) The first case of a *Staphylococcus pseudintermedius* infection after joint prothesis implantation in a dog. Pol J Microbiol 59: 133-135.

Oliveira NE, Cardozo AP, Marques Ede A, dos Santos KR, Giambiagi-de Marval M (**2007**) Interpretive criteria to differentiate low-and high-level mupirocin resistance in *Staphylococcus aureus*. J Med Microbiol 56: 937-939.

Oliveira LC, Leite CA, Brilhante RS, Carvalho CB (**2008**) Comparative study of the microbial profile from bilateral canine otitis externa. Can Vet J 49: 785-788.

Perreten V, Kadlec K, Schwarz S, Andersson UG, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L (**2010**) Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother 65: 1145-1154.

Prescott JF, Hanna WJ, Reid-Smith R, Drost K (**2002**) Antimicrobial drug use and resistance in dogs. Can Vet J 43: 107-116.

Ruscher C, Lubke-Becker A, Wleklinski CG, Soba A, Wieler LH, Walther B (**2009**) Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. Vet Microbiol 136: 197-201.

Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K (**2007**) Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol 45: 1118-1125.

Strommenger B, Kettlitz C, Werner G, Witte W (**2003**) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J Clin Microbiol 41: 4089-4094.

Tanner MA, Everett CL, Youvan DC (**2000**) Molecular phylogenetic evidence for noninvasive zoonotic transmission of *Staphylococcus intermedius* from canine pet to a human. J Clin Microbiol 38: 1628-1631.

van Duijkeren E, Houwers DJ, Schoormans A, Broekhuizen-Stins MJ, Ikawaty R, Fluit AC, Wagenaar JA (**2008**) Transmission of methicillin-resistant *Staphylococcus intermedius* between humans and animals. Vet Microbiol 128: 213-215.

Vanni M, Tognetti R, Pretti C, Crema F, Soldani G, Meucci V, Intorre L (**2009**) Antimicrobial susceptibility of *Staphylococcus intermedius* and *Staphylococcus schleiferi* isolated from dogs. Res Vet Sci 87: 192-195.

Vengust M, Anderson ME, Rousseau J, Weese JS (**2006**) Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Lett Appl Microbiol 43: 602-606.

Wakita Y, Kawano J, Shimizu A, Hajek V, Tomisaka E, Yasuda R, Matsudo E (**2002**) Development of a PCR test for the identification of *Staphylococcus intermedius* based on the 16S rDNA sequence. J Vet Med Sci 64: 603-605.

Wettstein K, Descloux S, Rossano A, Perreten V (**2008**) Emergence of methicillin-resistant *Staphylococcus pseudintermedius* in Switzerland: three cases of urinary tract infections in cats. Schweiz Arch Tierheilkd 150: 339-343.

Zubeir IE, Kanbar T, Alber J, Lämmler C, Akineden O, Weiss R, Zschöck M (**2007**) Phenotypic and genotypic characteristics of methicillin/oxacillin-resistant *Staphylococcus intermedius* isolated from clinical specimens during routine veterinary microbiological examinations. Vet Microbiol 121: 170-176.