AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Pregnancy and conception rate after two intravaginal inseminations with dog semen frozen either with 5% glycerol or 5% ethylene glycol

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/103794> since 2015-07-27T13:11:25Z

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in

Theriogenology, 75 (1), 2011 doi: 10.1016/j.theriogenology.2010.07.016.
<http://www.sciencedirect.com/science/article/pii/S0093691X1000409>

You may download, copy and otherwise use the AAM for non-commercial purposes provided that

your license is limited by the following restrictions:

(1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.

(2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.

(3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>),

<http://dx.doi.org/10.1016/j.amc.2013.11.030>

25

26

Isolation of methicillin-resistant *Staphylococcus pseudintermedius* from breeding dogs

Ada Rota^{a*}, Chiara Milani^b, Ilenia Drigo^c, Michele Drigo^d, Michela Corrò^c

^aDipartimento di Patologia Animale, Facoltà di Medicina Veterinaria, via Leonardo da Vinci 44, 10090 Grugliasco (TO), Italy e-mail: ada.rota@unito.it

^bDipartimento di Scienze Cliniche Veterinarie, Facoltà di Medicina Veterinaria, Agripolis, 35020 Legnaro (PD), Italy e-mail: chiara.milani@unipd.it

^cIstituto Zooprofilattico Sperimentale delle Venezie, Agripolis, 35020 Legnaro (PD), Italy e-mail: mcorro@izsvenezie.it

^dDipartimento di Sanità Pubblica, Patologia Comparata ed Igiene Veterinaria, Facoltà di Medicina Veterinaria, Agripolis, 35020 Legnaro (PD), Italy e-mail: michele.drigo@unipd.it

*Corresponding author

Tel + 39 11 6709051

Fax +39 11 6709097

e-mail:

ada.rota@unito.it

Abstract

The overuse of antimicrobials can select resistant bacteria strains; staphylococci have the ability to become resistant to all beta-lactam antimicrobials and are a significant concern in human medicine and a growing issue for veterinary medicine. Since in breeding kennels antimicrobials are sometimes incorrectly used, the objective of the work was to assess the occurrence of methicillin-resistant coagulase-positive staphylococci in breeding dogs. The research was carried out in 13 kennels that were allotted to three categories according to the intensity of antimicrobial use. Vaginal and milk swabs were taken from 87 healthy bitches around parturition and also from multiple organs of 27 of their pups that died within the first 2 weeks. Standard bacteriological examinations were carried out and coagulase-positive staphylococci were identified. All the coagulase-positive staphylococci resulted to be *Staphylococcus pseudintermedius*. Susceptibility to oxacillin and the presence of the *mecA* gene were tested. Nine out of 89 strains (six isolated from the bitches' milk and three from dead puppies, all belonging to kennels characterized by an excessive use of antimicrobials) were multidrug-resistant, methicillin-resistant and *mecA* positive. Our results confirm that excessive use of antimicrobials entails the risk of selecting resistant staphylococci strains. Our data also indicate that the bacterial flora of healthy dogs belonging to specific populations may act as a reservoir of resistance genes.

Keywords: *Staphylococcus pseudintermedius*; methicillin-resistance; *mecA* gene; dog

1. Introduction

Coagulase-positive staphylococci, (*Staphylococcus aureus*, *S. intermedius*, *S. pseudintermedius* and *S. delphini*) are commensal bacteria and opportunistic pathogens in most animal species [1]. Resistance to methicillin appeared in *S. aureus* in 1960 [2] and originated because of widespread use of beta-lactam antibiotics in nosocomial settings; the frequency of infections due to methicillin-resistant *S. aureus* has steadily increased over the years, representing a serious problem in human medicine [3]. Resistance to methicillin is due to the presence of the *mecA* gene, which encodes the altered penicillin-binding protein 2a (PBP2a) that has a reduced affinity for all beta-lactam antimicrobials; the *mecA* gene is carried on a mobile DNA element, the staphylococcal cassette chromosome *mec* (SCC*mec*) [4].

The most prevalent coagulase-positive staphylococcal species in dogs, isolated both from normal skin and oronasal mucosa and from skin and ear infections [5,6], was *S. intermedius*. Recently, using a molecular identification approach, canine isolates that had previously been phenotypically identified as *S. intermedius* were reclassified as *S. pseudintermedius* [7]. Devriese et al. [8] proposed that canine strains identified by traditional means should be reported as *S. pseudintermedius* unless shown by genomic investigation to belong to other related species. A small proportion of *S. intermedius* (old classification) strains isolated both from healthy dogs [9,10] and from dogs showing skin and ear infections [6] has proven to be methicillin-resistant *mecA* positive. Although *S. intermedius* (old classification) is seldom responsible of human infections, transfer from dog to human is possible [11,12].

Since antimicrobials are sometimes misused in breeding kennels, the aims of this work were 1) to assess the occurrence of methicillin-resistant coagulase-positive

staphylococci in breeding dogs; and 2) to compare the frequency of isolation of resistant strains among kennels differing in the use of antimicrobials.

2. Materials and Methods

2.1 Animals and sampling

Eighty-seven breeding bitches housed in 13 kennels located in Northern Italy were included in the study. The history of the kennels reported a different use/misuse of antibiotics, in particular around parturition, and they were consequently divided into three categories: 1) administration of amoxicillin or amoxicillin-clavulanic acid only when required by pathological conditions (5 kennels, 36 bitches); 2) almost routine administration of amoxicillin or amoxicillin-clavulanic acid around parturition (5 kennels, 29 bitches); 3) routine administration of various antimicrobial agents around parturition (II and III generation cephalosporins, macrolids, adopted after amoxicillin and amoxicillin-clavulanic acid had become ineffective) (3 kennels, 22 bitches).

Vaginal swabs were taken from the bitches 7-10 days before parturition and milk samples were collected 7-10 days after parturition; the bitches belonging to kennels of category II and III were under antimicrobial treatment when milk cultures were carried out. A guarded sterile swab (Copan Innovation[®], Brescia, Italy) was introduced into the cranial vagina after thorough disinfection of the vulva with a povidone-iodine solution, and placed in the Amies transport medium provided with the swab. A drop of milk was collected on a sterile swab (Copan Innovation[®], Brescia, Italy) from each of the caudal mammary glands, after local disinfection. A complete post-mortem examination was performed, including culture (from brain, stomach, intestine, liver, heart, lungs) and sensitivity testing, on puppies that died within the first 2 weeks. All the bitches, aged 1.5-9 years and belonging to different breeds, were in good health at the moment of

sampling and no signs or symptoms of either mastitis or metritis were present during the period of observation.

2.2 Isolation of coagulase-positive Staphylococci

Swabs were inoculated onto Blood Agar Base n°2 (Oxoid, Basingstoke, United Kingdom) with 5% defibrinated sheep blood (DiaTech, Jesi, Italy) and streaked out to obtain single colonies. After 24 hrs of incubation at 37 ± 1 °C in aerobic conditions, presumptive identification of staphylococci was based on colony morphology, Gram stain appearance, catalase test, haemolysis, pigment production. Coagulase-positive staphylococci were identified by coagulase reaction on rabbit plasma (Istituto Zooprofilattico delle Venezie, Legnaro, Italy) and clumping factor by Slidex Staph (BioMérieux, Marcy l'Etoile, France). API Staph ID 32 phenotypic test (BioMérieux, Marcy l'Etoile, France) and a specific PCR analysis for *S. aureus* [13] were also carried out.

2.3 Identification of *S. pseudintermedius*

S. pseudintermedius identification was performed according to Bannoehr et al. [14]. One colony of each isolate was suspended in 1 ml Phosphate Buffered Saline (pH 7.4) and DNA was extracted using DNeasy Blood & Tissue Kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions for Gram positive bacteria. The amplification was performed in a total volume of 50 µl containing 5 µl of DNA, 1.5 mM of $MgCl_2$, 0.1 U/µl of Fast Start Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany), 200 µM of each dNTPs (Applied Biosystems, Foster City, USA) and 0.5 µM of primers pta_f1 and pta_r1 (Table 1) [14]. DNA amplification was carried out in an Eppendorf Mastercycler Ep Gradient S (Eppendorf, Milano, Italy) with the following thermal cycling conditions: initial denaturation at 95 °C for 4 min, followed

by 40 cycles of amplification, denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 60 s, ending with a final elongation step of 5 min at 72 °C. After amplification, 10 µl of PCR products were subjected to enzymatic restriction with 1.5 U/µl of *Sau3AI* for 1h at 37 °C and the restriction products subsequently subjected to agarose gel electrophoresis. Restriction of *S. pseudintermedius* amplicons resulted in two fragment of 213 pb and 107 pb.

2.4 Antimicrobial susceptibility

Susceptibility to a panel of sixteen antimicrobial agents was determined by the disk diffusion method in Mueller-Hinton agar (Sclavo Diagnostics International, Siracusa, Italy) according to the guidelines of the Clinical Laboratory Standards Institute, when available [15, 16].

Discs of penicillin G (10 IU), ampicillin (10 µg), amoxicillin-clavulanic acid (20+10 µg) gentamicin (10 µg), oxacillin (1 µg), cefalexin (30 µg), cefuroxime (30 µg), spiramycin (100 µg), streptomycin (10 µg), tetracycline (30 µg), tilmicosin (15 µg), tylosin (30 µg), enrofloxacin (5 µg), rifampin (5 µg), tiamulin (30 µg), trimethoprim-sulfamethoxazole (1.25+23.75 µg) were tested. Interpretative criteria for the inhibition zone diameters of tylosin, spiramycin and streptomycin were given by the manufacturers.

Bacteria strains were stored at -80 °C and the susceptibility test was repeated after species identification.

2.5 Oxacillin resistance confirmation

Coagulase-positive staphylococci strains were transferred onto a selective medium, Oxacillin Resistance Screening Agar Base with selective supplement (Oxoid,

Basingstoke, United Kingdom) (oxacillin concentration: 2µg/ml), and incubated at 37°C±1°C for 24 h to confirm susceptibility to oxacillin, as a surrogate for methicillin.

2.6 *MecA* gene

Coagulase-positive staphylococci strains were tested for the presence of the *mecA* gene by PCR, using the primers reported in Table 1 [17]. DNA was extracted from each sample as previously described. The amplifications were performed in a total volume of 25 µl containing 5 µl of DNA, 3 mM MgCl₂, 0.05 U/µl FastStart Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany), 200 µM of each dNTPs (Applied Biosystems, Foster City, USA) and 0.5 µM of primer. DNA amplification was carried out in an Eppendorf Mastercycler Ep Gradient S (Eppendorf, Milano, Italy) with the following thermal cycling conditions: initial denaturation at 95 °C for 6 min, followed by 30 cycles of amplification, denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, ending with a final elongation step of 5 min at 72 °C. After amplification, 10 µl of PCR products were subjected to agarose gel electrophoresis.

2.7 Statistical analysis

The frequency of isolation of coagulase-positive staphylococci, both as a whole and in each of the two localisations, was compared among the three categories of breeding kennels by using chi-squared analysis. The frequency of isolation of methicillin-resistant coagulase-positive staphylococci strains was analyzed using the Fisher exact test, considering kennels of category 1 and 2 as a unique category. A P value < 0.05 was considered significant.

3. Results

A total number of 76 coagulase-positive staphylococci strains were isolated from the bitches (33 from vagina and 43 from milk) and 13 coagulase-positive staphylococci strains were isolated from 27 dead puppies.

All the coagulase-positive staphylococci resulted to be *S. pseudintermedius*.

The frequency of *S. pseudintermedius* colonization was not significantly different among the three categories of kennels, neither as a whole nor in each of the two localisations. In 7 out of 13 cases of dead puppies, *S. pseudintermedius* had caused septicemia: in six cases it was also the only bacterium isolated from the milk of the corresponding dams, and in one case it was isolated from the dam's vagina.

All the isolated *S. pseudintermedius* strains showed high percentages of resistance to different antimicrobials, particularly to penicillin and ampicillin, spiramycin and tetracycline (Table 2).

Eight strains isolated from the bitches' milk and three from the dead pups were resistant to oxacillin; seven of the bitch strains and all the pup strains confirmed the resistance in the selective medium, with the higher oxacillin concentration. Six out of seven strains isolated from the bitches and all three strains isolated from the dead pups carried the *mecA* gene. In the *mecA* negative bitch strain, either *mecA* was not present or mutations in the primer region may have occurred.

The origin, antibiotic resistance characteristics and presence/absence of the *mecA* gene in the methicillin-resistant strains are reported in Table 3. All these strains show multidrug resistance. All the isolates shown in Table 3 came from two kennels belonging to category 3. The isolates with numbers 6 and 7 came from two bitches, while those bearing numbers 8 and 9 derived from their dead puppies. Overall, the prevalence of *mecA* positive methicillin-resistant strains was 7.9% in the bitches; the percentage of *mecA* positive methicillin-resistant strains on all *S. pseudintermedius* isolates was 10.1%.

Table 4 shows the number of methicillin-resistant *mecA* positive *S. pseudintermedius* strains in the three categories of kennels and in the two localizations of the bitches. The frequency of isolation of methicillin-resistant *mecA* positive *S. pseudintermedius* strains in the bitches was higher in kennels belonging to category 3 (χ^2 [Yates corrected]=12.48; P<0.001).

All the staphylococci strains that resulted susceptible to oxacillin by disk diffusion test confirmed their susceptibility in the selective medium and resulted *mecA* negative.

4. Discussion

Antimicrobials are sometimes misused in breeding kennels and different agents may be routinely administered around parturition to prevent neonatal infections, instead of adopting more appropriate measures to minimise structural predisposing factors [18].

By comparing the antibiotic resistance in the intestinal flora, De Graef et al. [19] found higher resistance percentages in dogs kept in breeding kennels than in privately owned animals; the same study described also a relation between the antimicrobials used and the resistances of bacteria, thus suggesting the possibility that resistance genes may be transferred among bacteria colonising group-housed dogs.

All the coagulase-positive staphylococci strains isolated from the bitches in our work were identified as *S. pseudintermedius* by molecular analysis, in agreement with recent taxonomy observations on canine isolates [1,7,8].

In our study, *S. pseudintermedius* showed high percentages of antimicrobial resistance: also when bitches were not exposed to antimicrobial treatment before sampling, as in kennels of category 1, mean resistance rates for penicillin, ampicillin and spiramycin were higher than 80%, for tetracycline higher than 25% and for streptomycin higher than 18%. In a recent work [20], the Authors found a widespread occurrence of resistance in *S. pseudintermedius* from skin and ear infections of dogs with no recent

history of antimicrobial treatment. The high genetic polymorphism of the isolates suggested the possible transfer of resistance genes among *S. pseudintermedius* strains as well as other bacteria in the dog population and it could be worth testing this hypothesis also in our experimental series.

The occurrence of methicillin-resistance in *S. pseudintermedius* isolated in two breeding kennels, both belonging to the group characterised by excessive antimicrobials use, confirms that antimicrobial overuse selects resistant strains. Administration of antimicrobial drugs, specifically β -lactams and fluoroquinolones, in the previous 90 days, resulted in a significant risk factor for methicillin-resistant *S. aureus* (MRSA) infection in dogs [21].

Methicillin-resistant *S. pseudintermedius* (MRSP) colonization of healthy dogs living in a confined environment raises a number of issues including a) possible horizontal transfer from dog to dog, and particularly between females and males; b) the pattern of colonization over time; c) health hazard for dogs within kennels; and d) potential health hazard for breeders. A recent study by Loeffler et al. [22] showed that MRSA did not transmit readily between apparently healthy dogs housed in shared kennels and that MRSA carriage was only transient in a regularly cleaned and disinfected environment. However, these observations should be confirmed for MRSP and, furthermore, any infection developed by MRSP-colonized animals, would probably be difficult to treat. In fact, MRSP isolates that are positive for the *mecA* gene are generally multiresistant [23], as in our case, although not necessarily more virulent than their methicillin-susceptible counterparts [24]. A longitudinal study might help assess the persistence of MRSP carriage and, consequently, the effective risk that a group-housed dog population may become a reservoir for resistance genes. However, if antimicrobial pressure is not reduced in the concerned kennels, other resistant strains are going to be selected.

S. intermedius (old classification) is not usually a human pathogen, although it is known to cause invasive infection following dog-bites [25]; sporadic cases of bacteraemia in patients who had undergone invasive procedures have been reported [26], one case of otitis externa [27] and, recently, a case of *S. pseudintermedius* catheter-related bacteraemia in a hemophilic child [28]. Transmission of *S. intermedius* (old classification) from dogs to their owners can occur [11,12] and it has already been described also for methicillin-resistant strains [29]. The occurrence of *S. intermedius* (old classification) in the owners of dogs affected by deep pyoderma was significantly higher than in the control group [12]. However a recent investigation showed that MRSP nasal colonization of the owners of dogs affected by MRSP skin infection was uncommon and transient [30]. Nasal carriage of MRSP was detected in one staff member of a veterinary teaching hospital [31]: personnel of veterinary clinics should be made aware of the risk of nosocomial transmission of MRSP.

Although MRSP strains are not alarming as zoonotic agents, they represent a serious concern as a potential source of horizontal gene transfer to other staphylococci, converting methicillin-susceptible *S. aureus* colonizing humans into MRSA through transfer of the mobile SCC containing the *mecA* gene [24,32]. *S. aureus* is indeed thought to have acquired resistance through horizontal gene transmission from coagulase-negative *S. sciuri* [33].

The prevalence of MRSP found in our study is higher than the values previously reported for healthy dogs (1.5% [10]; 2% [9]) and is similar to data (6.3%) reported in clinical samples of diseased dogs [10,34]. However, in our work, the frequency is biased in kennels where the excessive use of antimicrobials has increased the selection of resistant strains.

Genetic characterization of our MRSP isolates would be of great epidemiological interest: their belonging to the multiresistant clonal lineage widespread in several

European countries (including Italy [35]), would confirm the spreading of MRSP strains with relatively stable clones. However, the assessment of the genetical profile of the isolates was out of the scope of this research.

A previous retrospective investigation on the bacteriological status of canine milk and septicaemia of neonatal puppies [36] excluded *S. intermedius* (old classification) as a major cause of septicaemia in neonates because, although isolated from the milk of the dams, the bacterium was never found in the organs of the septicaemic puppies. This issue deserves further investigation. In our work, *S. pseudintermedius* was isolated from the organs of septicaemic puppies and it was also the only bacterium isolated from the milk of the corresponding dams or from the dams' vagina. Two of the milk and puppy isolates were methicillin-resistant *mecA* positive. Although the isolates were not typed, a link between the strains from dam and puppy is very likely.

Our results confirm that the misuse of antimicrobials may lead to serious consequences because resistant strains may become a health hazard in veterinary and human medicine; methicillin-resistant staphylococcal infections may become causes of morbidity and mortality in companion animals but domestic animals may also represent a reservoir of infection and a zoonotic source [1]. MRSP should not only be considered as a nosocomial pathogen in veterinary settings [35] but also as a potentially problematic commensal in particular dog populations.

Acknowledgments

The authors thank Roberto Perin and Anna Sturaro for technical assistance.

References

- [1] Weese JS, van Duijkeren E. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol 2010; 140: 418-29.
- [2] Jevons MP, Coe AW, Parker MT. Methicillin resistance in staphylococci. Lancet 1963; 1: 904-7.
- [3] Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 2008; 46 (Suppl. 5): 344-9.
- [4] Lin AE, Davies JE. Occurrence of highly fluoroquinolone-resistant and methicillin-resistant *Staphylococcus aureus* in domestic animals. Can J Microbiol 2007; 53: 925-9.
- [5] Allaker RP, Jensen L, Lloyd DH, Lamport AI. Colonization of neonatal puppies by staphylococci. Brit Vet J 1992; 148: 523-8.
- [6] Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JML, Winkler M, Weiss R, Lloyd DH. First report of multiresistant, *mecA*-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Vet Dermatol 2007; 18: 412-21.
- [7] Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J Clin Microbiol 2007; 45: 2770-8.
- [8] Devriese LA, Hermans K, Baele M, Haesebrouck F. *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius*. Vet Microbiol 2009; 133: 206-7.
- [9] Vengust M, Anderson M, Rousseau J, Weese J. Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Lett Appl Microbiol 2006; 43: 602-6.
- [10] Griffeth GC, Morris DO, Abraham JL, Shofer FS, Rankin SC. Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. Vet Dermatol 2008; 19:142-9.
- [11] Harvey RG, Marples RR, Noble WC. Nasal carriage of *Staphylococcus intermedius* in humans in contact with dogs. Microb Ecol 1994; 7: 225-7.
- [12] Guardabassi L, Loeber ME, Jacobson A. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Vet Microbiol 2004; 98: 23-7.
- [13] Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxin, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000; 38: 1032-5.

- [14] Bannoehr J, Franco A, Iurescia M, Battisti A, Fitzgerald JR. Molecular diagnostic identification of *Staphylococcus pseudintermedius*. J Clin Microbiol 2009; 47: 469-71.
- [15] Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard- Third Edition (M31-A3), Wayne, PA, 2008.
- [16] Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement (M100-S19), Wayne, PA, 2009.
- [17] Louie L, Goodfellow J, Mathieu P, Glatt A, Louie M, Simor AE. Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. J Clin Microbiol 2002; 40: 2786-90.
- [18] Sager M, Remmers C. Some aspects of perinatal mortality in the dog. A clinical, bacteriological and pathological study [in German]. Tierärztl Prax 1990; 18: 415-9.
- [19] De Graef EM, Decostere A, Devriese LA, Haesebrouck F. Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. Microb Drug Resist 2004; 10: 65-9.
- [20] Norström M, Sunde M, Tharaldsen H, Mørk T, Bergsjø B, Kruse H. Antimicrobial resistance in *Staphylococcus pseudintermedius* in the Norwegian dog population. Microb Drug Resist 2009; 15: 55-9.
- [21] Faires MC, Traverse M, Tater KC, Pearl DL, Weese JS. Methicillin-resistant and -susceptible *Staphylococcus aureus* infections in dogs. Emerg Infect Dis 2010; 16: 69-75.
- [22] Loeffler A, Pfeiffer DU, Lindsay JA, Soares-Magalhaes R, Lloyd DH. Lack of transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) between apparently healthy dogs in a rescue kennel. Vet Microbiol 2010; 141: 178-81.
- [23] Gortel K, Campbell KL, Kakoma I, Whittem T, Schaeffer DJ, Weisiger RM. Methicillin resistance among staphylococci isolated from dogs. Am J Vet Res 1999; 60: 1526-30.
- [24] Cohn LA, Middleton JR. A veterinary perspective on methicillin-resistant staphylococci. J Vet Emerg Crit Care (San Antonio) 2010; 20: 31-45.
- [25] Talan DA, Staats D, Staats A, Goldstein EJ, Singer K, Overturf GD. *Staphylococcus intermedius* in canine gingival and canine-inflicted human wound infections: laboratory characterization of a newly recognized zoonotic pathogen. J Clin Microbiol 1989; 27: 78-81.
- [26] Vandenesch F, Célaré M, Arpin D, Bes M, Greenland T, Etienne J. Catheter-related bacteremia associated with coagulase-positive *Staphylococcus intermedius*. J Clin Microbiol 1995; 33: 2508-10.

- [27] Tanner MA, Everett CL, Youvan DC. Molecular phylogenetic evidence for noninvasive zoonotic transmission of *Staphylococcus intermedius* from a canine pet to a human. J Clin Microbiol 2000; 38: 1628–31.
- [28] Chuang C-Y, Yang Y-L, Hsueh P-R, Lee P-I. Catheter-related bacteremia caused by *Staphylococcus pseudintermedius* refractory to antibiotic-lock therapy in a hemophilic child with dog exposure. J Clin Microbiol 2010; 48: 1497-8.
- [29] van Duijkeren E, Houwers DJ, Schoormans A, Broekhuizen-Stins MJ, Ikawaty R, Fluit, Wagenaar JA. Transmission of methicillin-resistant *Staphylococcus intermedius* between humans and animals. Vet Microbiol 2008; 128: 213-5.
- [30] Frank LA, Kania SA, Kirzeder EM, Eberlein LC, Bemis DA. Risk of colonization or gene transfer to owners of dogs with methicillin-resistant *Staphylococcus pseudintermedius*. Vet Dermatol 2009; 20: 496-501.
- [31] Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol 2007; 45: 1118-25.
- [32] Hanssen AM, Kjeldsen G, Ericson Sollid JU. Local variants of staphylococcal cassette chromosome mec in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci: evidence of horizontal gene transfer? Antimicrob Agents Chemoter 2004; 48: 285-96.
- [33] Wu S, Piscitelli C, de Lencastre H, Tomasz A. Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of mecA from a methicillin susceptible strain of *Staphylococcus sciuri*. Microb Drug Resist 1996, 2, 435-41.
- [34] Ruscher C, Lübke-Becker A, Wleklinski CG, Soba A, Wieler LH, Walther B. Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidae. Vet Microbiol 2009; 136: 197-201.
- [35] Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, 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. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother 2010; 65: 1145-54.
- [36] Schäfer-Somi S, Spargser J, Breitenfellner J, Aurich JE. Bacteriological status of canine milk and septicaemia in neonatal puppies--a retrospective study. J Vet Med B Infect Dis Vet Public Health 2003; 50: 343-6.

439 Table 1. Primers used to identify *Staphylococcus pseudintermedius* and to detect the presence of the *mecA* gene

440

441

Primers	Sequence 5'-3'	Product size (bp)	Reference
Pta_f1	AAA GAC AAA CTT TCA GGT AA	320	[14]
Pta_r1	GCA TAA ACA AGC ATT GTA CCG		
<i>mecA</i> _F	AAA ATC GAT GGT AAA GGT TGG C	533	[16]
<i>mecA</i> _R	AGT TCT GCA GTA CCG GAT TTG C		

442

443 Table 2. Percentages of resistance to different antimicrobials of *Staphylococcus pseudintermedius* strains isolated in two localisations from bitches
 444 housed in three categories of kennels

Kennel category	1		2		3	
Origin^a	M	V	M	V	M	V
N° of isolates	21	13	9	12	13	8
Penicillin	85.7	92.3	88.9	100	100	100
Ampicillin	80.9	92.3	88.9	100	100	100
Amoxycillin-clavulanic acid	0	0	0	0	53.8	12.5
Oxacillin	0	0	0	0	61.5	0
Cefalexin	4.8	0	0	0	38.5	0
Cefuroxime	4.8	0	0	0	38.5	0
Spiramycin	90.5	69.2	88.5	90.0	100	100
Rifampin	4.8	0	0	0	0	0
Tilmicosin	0	7.7	0	10	84.6	12.5
Tylosin	0	15.4	22.2	30.0	100	37.5
Tetracycline	23.8	30.8	44.4	60.0	84.6	62.5
Streptomycin	14.3	23.1	55.6	30.0	92.3	37.5
Gentamycin	0	0	0	0	38.5	0
Enrofloxacin	0	0	0	0	53.8	0
Trimethoprim-sulfamethoxazole	9.5	15.4	11.1	20.0	84.6	12.5
Tiamulin	4.8	0	11.1	0	0	0

445 ^aM, milk; V, vagina.

448 Table 3. Antibigrams of methicillin-resistant *Staphylococcus pseudintermedius* isolates. Dams and corresponding puppies are linked with brackets.

Strain	1	2	3	4	5	6	7	8	9	10
Origin ^a	M	M	M	M	M	M	M	P	P	P
Kennel category	3	3	3	3	3	3	3	3	3	3
Penicillin	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	R	R
Amoxycillin-clavulanic acid	R	R	R	R	R	R	R	R	R	R
Oxacillin	R	R	R	R	R	R	R	R	R	R
Cefalexin	R	I	R	S	R	R	R	R	R	R
Cefuroxime	R	R	R	S	R	R	R	R	R	R
Spiramycin	R	R	R	R	R	R	R	R	R	R
Rifampin	S	S	S	S	S	S	S	S	S	S
Tilmicosin	R	R	R	R	R	R	R	R	R	R
Tylosin	R	R	R	R	R	R	R	R	R	R
Tetracycline	R	R	S	I	R	R	R	R	R	R
Streptomycin	R	R	R	R	R	R	R	R	R	R
Gentamycin	R	I	R	I	R	I	R	R	R	R
Enrofloxacin	R	R	R	R	R	R	R	R	R	R
Trimethoprim-sulfamethoxazole	R	R	R	R	R	R	R	R	R	R
Tiamulin	S	S	S	S	S	S	S	S	S	S
<i>mecA</i>	+	+	+	+	-	+	+	+	+	+

449 ^aM, milk; P, dead pup. R, resistant; S, susceptible; I, intermediate

450

451 Table 4. *Staphylococcus pseudintermedius* colonisation of the vagina and milk of the bitches belonging to the three categories of kennels (1= low,
452 correct use of antimicrobials ; 2= moderate; 3= excessive); occurrence of methicillin-resistant *S. pseudintermedius* (MRSP) strains.

Kennel category	1	2	3
N of bitches	36	29	22
Percentage and (N) of bitches colonised by <i>S. pseudintermedius</i> in vagina and/or milk	66.7 (24)	65.5 (19)	77.3 (17)
Percentage and (N) of vaginal isolates	36.1 (13)	41.4 (12)	36.4 (8)
Percentage and (N) of milk isolates	52.8 (19)	37.9 (11)	59.1 (13)
Total N of isolates	32	23	21
Percentage and (N) of MRSP on vaginal isolates	0 (0/13)	0 (0/12)	0 (0/8)
Percentage and (N) of MRSP on milk isolates	0 (0/19)	0 (0/11)	46.1 (6/13)

453

454