Characterisation of clinical canine meticillin-resistant and meticillin-susceptible Staphylococcus pseudintermedius in France

Marisa Haenni, Natasha Alves de Moraes, Pierre Châtre, Christine Médaille, Arshnee Moodley, Jean-Yves Madec

Journal of Global Antimicrobial Resistance 2 (2014) 119–123

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Characterisation of clinical canine meticillin-resistant and meticillin-susceptible *Staphylococcus pseudintermedius* in France

Marisa Haenni [a,*], Natasha Alves de Moraes [a], Pierre Châtre [a], Christine Médaille [b], Arshnee Moodley [c], Jean-Yves Madec [a]

[a] *Agence Nationale de Sécurité Sanitaire (Anses), Unité Antibiorésistance et Virulence Bactériennes, Lyon, France*
[b] *Vebio, Veterinary Laboratory, Arcueil, France*
[c] *Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

ARTICLE INFO

Article history:
Received 17 July 2013
Received in revised form 4 February 2014
Accepted 24 February 2014

Keywords:
Dog
Antibiotic resistance
MLST
PFGE
Spa

ABSTRACT

Staphylococcus pseudintermedius is a frequent pathogen in dogs. The emergence of meticillin-resistant *S. pseudintermedius* (MRSP), which is concomitantly resistant to nearly all veterinary licensed antibiotics used for systemic treatment in dogs, is a major problem for veterinarians. In France, 16.9% (41/243) of the *S. pseudintermedius* collected in 2010 were MRSP. They mainly belonged to the multiresistant MLST sequence type ST71, *spa* type t02, SCC*mec* type II-III (ST71–t02–II-III) European clone. Moreover, we also report the emergence of multiresistant meticillin-susceptible *S. pseudintermedius* isolates presenting atypical and/or new *spa* types. This study highlights the need for surveillance, optimised treatment guidelines and new therapeutic alternatives.

1. Introduction

Staphylococcus pseudintermedius, initially described in 2005, is a commensal in dogs and is also recognised as a frequent opportunistic pathogen, primarily causing skin infections and post-operative complications. Moreover, whilst its natural host is dogs, and to a lesser extent cats, human carriage or infections, which are typically associated with exposure to dogs, have been reported, highlighting the zoonotic potential of *S. pseudintermedius* [1].

Initially, *S. pseudintermedius* was mostly susceptible to antibiotics and was not a particular issue for veterinarians. However, since 2006 meticillin-resistant *S. pseudintermedius* (MRSP) has emerged as a pathogen resistant to nearly all classes of antibiotics available for use in dogs [2] and represents a threat to small animal antibiotic therapy owing to limited treatment options. An international multicentre study showed that a few distinct MRSP clones are broadly disseminated, including multilocus sequence typing (MLST) sequence type ST71, *spa* type t02, staphylococcal cassette chromosome *mec* (SCC*mec*) type II-III (ST71–t02–II-III) in Europe and ST68–t06–V in the USA, whilst recent data indicate the emergence of geographic-specific genotypes, e.g. ST71–t06–II-III in France or ST106 in Norway [3,4]. Recent efforts have focused on understanding MRSP clonality and spread, but very little is known about the genetic diversity of meticillin-susceptible *S. pseudintermedius* (MSSP) [2,5–7].

To gain insight into the population structure of *S. pseudintermedius* infecting dogs in France, a 1-year survey was conducted to investigate the antimicrobial susceptibility patterns and genetic diversity among MRSP and MSSP clinical isolates.

2. Materials and methods

2.1. Bacterial sampling and identification

Between January and December 2010, 268 coagulase-positive staphylococci (CoPS) were isolated from diseased dogs visiting a referral veterinary clinic in Paris, France. This clinic receives isolates from all geographical regions throughout France. Each clinical isolate corresponded to a unique animal. All isolates were later sent to the Anses laboratory in Lyon (France) for further analysis. Species identification was performed by *kat* restriction fragment length polymorphism (RFLP) [8], and all isolates identified as *Staphylococcus intermedius* were further confirmed using a multiplex *nuc* PCR as described by Sasaki et al. [9].

* Corresponding author. Tel.: +33 4 7869 6830; fax: +33 4 7861 9145.
E-mail address: marisa.haenni@anses.fr (M. Haenni).

http://dx.doi.org/10.1016/j.jgar.2014.02.002
2213-7165/

2.2. Antimicrobial susceptibility tests

All *S. pseudintermedius* isolates were tested for their antimicrobial susceptibility by the disc diffusion method according to the guidelines of the French Society for Microbiology (CA-SFM) (http://www.sfm-microbiologie.fr). Antibiotics tested were oxacillin 5 μg (breakpoints, susceptible ≥20 mm/resistant <20 mm), penicillin 6 μg, fusidic acid 10 μg, kanamycin 30 μg, gentamicin 15 μg, tobramycin 10 μg, erythromycin 15 μg, spiramycin 100 μg, lincomycin 15 μg, pristinamycin 15 μg, tetracycline 30 μg, chloramphenicol 30 μg, florfenicol 30 μg, enrofloxacin 5 μg, vancomycin 30 μg and teicoplanin 30 μg (Mast Diagnostics, Amiens, France). The breakpoints used are shown in Table 1, and bacteria were classified as susceptible, intermediate or resistant according to the clinical breakpoints approved by the CA-SFM. *Staphylococcus aureus* ATCC 25923 was used as the quality control strain.

2.3. Detection and molecular characterisation of S. pseudintermedius

PCR screening for *mecA* was systematically performed on all *S. pseudintermedius* isolates. Sequencing of the variable region X of the *S. pseudintermedius spa* gene was performed on all isolates according to Moodley et al. [10], and new *spa* types were assigned by Arshnee Moodley, the curator of the *spa* type database (asm@sund.ku.dk). Pulsed-field gel electrophoresis (PFGE) was performed on all MRSP isolates using the *Sma*I restriction enzyme according to a previously published method with slight modifications (pulse time of 1–30 s and total run time of 23 h) [11]. Cluster analysis was performed by UPGMA (unweighted pair-group method with arithmetic mean) based on the Dice similarity coefficient, with optimisation and position tolerance set at 0.5% and 1%, respectively. Isolates were clustered using a 73% similarity cut-off, above which isolates were considered to be closely related and assigned to the same PFGE type. This similarity cut-off value allowed discrimination between ST71 and non-ST71 isolates.

Based on the PFGE cluster analysis, MLST was performed on 20 MRSP isolates according to the recently published seven-locus scheme [12]. Sequence types were determined using the MLST website (http://pubmlst.org/spseudintermedius) and new sequence types were assigned by the curator Vincent Perreten (vincent.perreten@vetsuisse.unibe.ch). SCC*mec* typing of all MRSP was performed by PCR according to published methods [13].

Table 1
Antimicrobial susceptibility by the disc diffusion method in meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP) and meticillin-resistant *S. pseudintermedius* (MRSP) isolates.

Antimicrobial agent	Breakpoints (mm) (S≥/R<)	MSSP (*n* = 202)	MRSP (*n* = 41)
		No. (%) resistant	No. (%) resistant
Penicillin	29/29	131 (64.9)	41 (100.0)
Fusidic acid	24/24	2 (1.0)	1 (2.4)
Lincomycin	21/17	52 (25.7)	38 (92.7)
Erythromycin	22/17	77 (38.1)	40 (97.6)
Spiramycin	20/20	77 (38.1)	40 (97.6)
Pristinamycin	22/19	0 (0.0)	0 (0.0)
Enrofloxacin	22/17	19 (9.4)	38 (92.7)
Kanamycin	17/15	88 (43.6)	40 (97.6)
Tobramycin	20/20	11 (5.4)	35 (85.4)
Gentamicin	20/20	11 (5.4)	32 (78.0)
Tetracycline	19/17	105 (52.0)	29 (70.7)
Vancomycin	17/17	0 (0.0)	0 (0.0)
Teicoplanin	17/17	0 (0.0)	0 (0.0)
Chloramphenicol	22/19	50 (24.8)	9 (22.0)
Florfenicol	N/A	N/I	N/I

S, susceptible; R, resistant; N/A, not available; N/I, not interpreted (due to lack of breakpoints).

3. Results

Among the 268 CoPS isolated at the referral clinic, 243 (90.7%) were identified as *S. pseudintermedius* and were further characterised. The 25 remaining isolates belonged to the species *S. aureus* (*n* = 14), *S. intermedius* (*n* = 3) and *Staphylococcus schleiferi* (*n* = 8). *S. pseudintermedius* was recovered from dogs suffering from skin infections (*n* = 135), otitis (*n* = 24), bone infections (*n* = 16), urinary tract infections (*n* = 18), genital tract infections (*n* = 3), respiratory diseases (*n* = 10), mouth infections (*n* = 3) and eye infections (*n* = 11); the pathology was unknown for 23 isolates. In total, 41 MRSP (16.9%) were identified, which were isolated from unrelated animals originating from 12 different French districts (Nord, *n* = 12; Paris, *n* = 11; Val-de-Marne, *n* = 5; Hauts-de-Seine, *n* = 3; Drôme, *n* = 2; Maine-et-Loire, *n* = 2; Côte d'Or, *n* = 1; Doubs, *n* = 1; Haute-Savoie, *n* = 1; Moselle, *n* = 1; Oise, *n* = 1; and Val d'Oise, *n* = 1).

Amongst the 202 MSSP, the top four resistance phenotypes were penicillin (64.9%), tetracycline (52.0%), kanamycin (43.6%), and erythromycin and spiramycin (38.1%). Approximately 25% of these isolates were also resistant to lincomycin and chloramphenicol. Multiresistance (resistance to at least three antibiotic classes) was detected in 30.7% of MSSP isolates (62/202), and the most prevalent association included resistance to penicillin, erythromycin, kanamycin and tetracycline (46/62; 74.2%). Ten MSSP isolates were additionally resistant to fluoroquinolones. Among the 41 MRSP isolates, 40 were multiresistant and presented quasi-systematic (>90%) co-resistances to erythromycin, spiramycin, lincomycin, enrofloxacin and kanamycin (Table 1; Fig. 1). Resistance to tobramycin, gentamicin and tetracycline was >70%, whereas resistance to chloramphenicol was 22.0%. One MRSP isolate was only resistant to tetracycline. Independent of the meticillin resistance, no resistance was observed to pristinamycin, vancomycin and teicoplanin. No apparent decreased susceptibility to florfenicol was recorded, since no bimodal distribution of the florfenicol inhibition zone diameters was observed for all isolates (diameters ranging from 26 mm to 37 mm).

Among MSSP, only 22.3% of isolates (45/202) could be typed by amplification and sequencing of the variable region X in the *spa* gene. Nineteen different *spa* types were observed, with t06 (*n* = 10) and t15 (*n* = 9) being the most common types. Twelve new *spa* types (t36 to t48) and four new repeats were identified. Amongst the MRSP, 35/41 (85.4%) were *spa* typeable. Five *spa* types were identified, with 27/41 (65.9%) of the isolates displaying *spa* type t02 (Fig. 1; Table 2).

Further characterisation of the MRSP isolates showed the prototypically SCC*mec* II-III cassette present in 36 isolates (87.8%), and the 5 remaining isolates harboured a type IV cassette. PFGE showed the presence of two main clusters, PFGE types A and B (Fig. 1). PFGE type A was further divided in two main subclusters, A1 and A2. Based on the PFGE profiles, 20 isolates were typed by MLST, including 13 isolates from PFGE type A (6 belonging to t02 and all non-t02 isolates) as well as all 7 isolates from PFGE type B. Those isolates with PFGE type A all belonged to ST71, whereas three isolates belonging to PFGE type B were ST258 and the remaining four type B isolates were new sequence types (namely ST193, ST194, ST293 and ST294). PFGE type B included six of the seven isolates that were either *spa* non-typeable or harboured an atypical *spa* type t41.

4. Discussion

This study confirms that *S. pseudintermedius* is the most frequent CoPS associated with staphylococcal infections in dogs, and more specifically with skin infections (135/243; 55.6%).

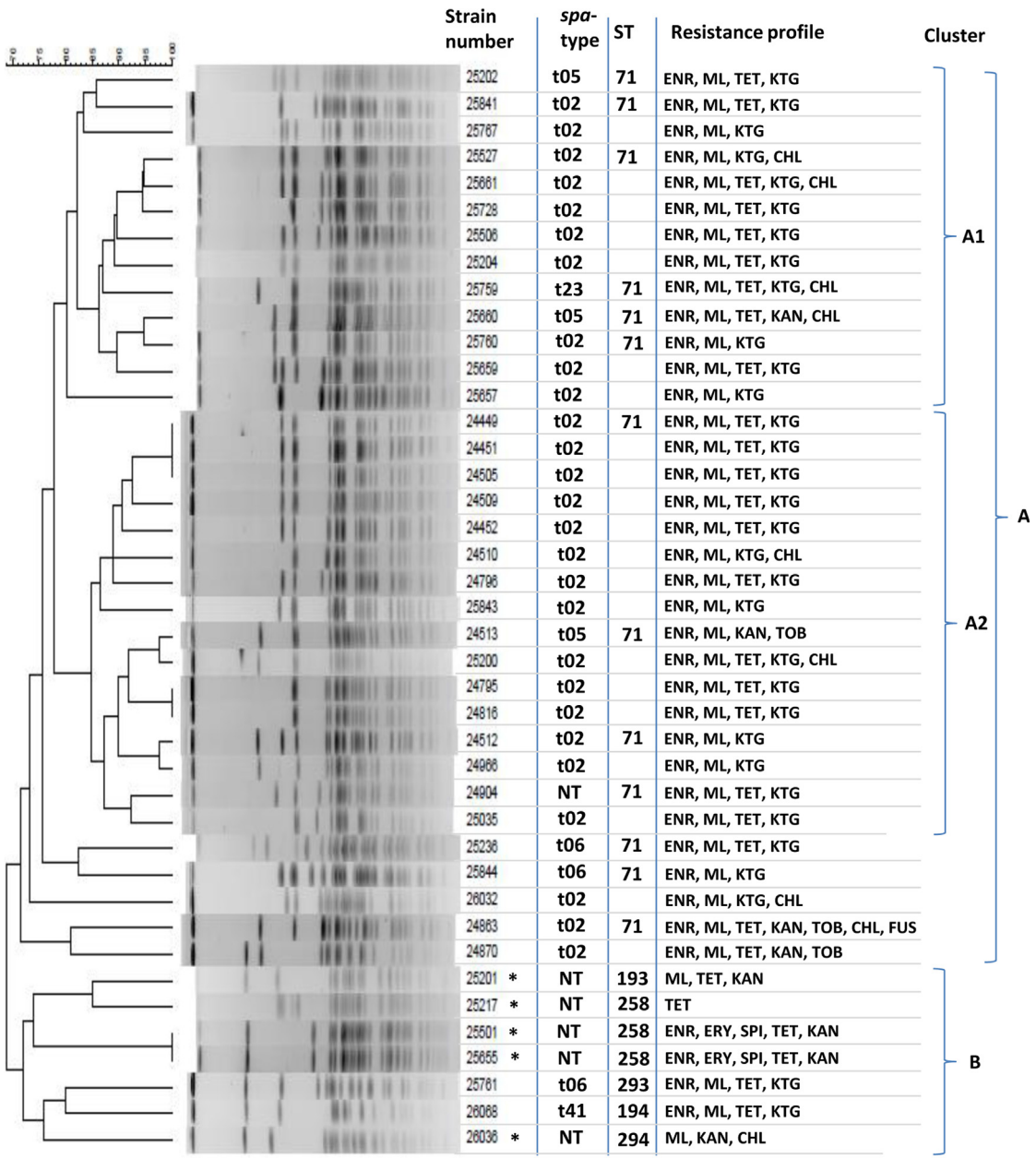

Fig. 1. Pulsed field gel electrophoresis (PFGE) patterns and typing characteristics of 41 meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates. All isolates harboured staphylococcal cassette chromosome *mec* (SCC*mec*) II-III, except for five isolates, which harboured SCC*mec* IV and are marked by an asterisk (*). ENR, enrofloxacin; ML, macrolides and lincosamides; TET, tetracycline; KTG, kanamycin/tobramycin/gentamicin; CHL, chloramphenicol; KAN, kanamycin; TOB, tobramycin; FUS, fusidic acid; ERY, erythromycin; SPI, spiramycin.

Among *S. pseudintermedius*, 16.9% (41/243) were MRSP and were associated with severe or recurrent infections that were unsuccessfully treated with antibiotics. This is higher than what has been described for infected dogs in Germany (7% in 2007 [14]; 8% in Hannover over the 2007–2009 time period [15]) and in North China (13% in 2008–2009 [16]) but is much lower than that recently reported in Southern China (48% in 2007–2009 [17]).

Whilst multiresistance was lower in MSSP compared with MRSP, these MSSP strains, which were isolated in 2010, were more resistant than strains isolated in France 8 years ago [18] and in other countries [5]. This indicates an overall increasing resistance in MSSP, and the emergence of multiresistant phenotypes may hamper future treatment possibilities.

MRSP were resistant to all classes of antibiotics used for systemic treatment in dogs (notably β-lactams, fluoroquinolones, tetracyclines and lincosamides), as is typically described for MRSP worldwide. Chloramphenicol is considered a therapeutic option; however, chloramphenicol-resistant MRSP and MSSP isolates do occur (ca. 25% in this study) and the use of this antibiotic in France is rare since it is restricted to topical formulations. Owing to limited treatment options, alternatives are desperately needed. For topical treatment, fusidic acid remains mostly active, and non-antibiotic therapies such as antiseptics (e.g. chlorhexidine) could also be used [1,19]. Moreover, bacteriophage therapy could also be an option in the future. In systemic infections, revision of current treatment guidelines, antibiotic stewardship, stringent infection control policies and evaluation of older antimicrobials, e.g. nitrofurantoin in urinary tract infections, would help prevent MRSP infections [20].

MRSP appear to have widely disseminated through two major clonal types: ST71–SCC*mec* type II-III in Europe and ST68–SCC*mec* type V in the USA. ST71 is frequently associated with *spa* type t02,

Table 2
spa types of meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP) and meticillin-resistant *S. pseudintermedius* (MRSP) isolates.

spa type	No. of strains	Repeats[a]
MRSP (n = 41)		
t02	27	r01 r02 r03 r03 r03 r06 r05
t05	3	r01 r02 r03 r03 r03 r03 r06 r05
t06	3	r01 r02 r03 r03 r06 r05
t23	1	r01 r09 r02 r02 r13 r03 r06 r05
t41	1	r01 r12 r02 r02 r03 r13 r02 r06 r05
NT	6	
MSSP (n = 202)		
t02	1	r01 r02 r03 r03 r03 r06 r05
t05	4	r01 r02 r03 r03 r03 r03 r06 r05
t06	10	r01 r02 r03 r03 r06 r05
t15	9	r01 r02 r03 r03 r03 r03 r03 r06 r05
t17	1	r01 r02 r03 r03 r03 r03 r03 r03 r06 r05
t18	1	r01 r02 r02 r03 r03 r03 r06 r05
t23	1	r01 r09 r02 r02 r13 r03 r06 r05
t36	2	r01 r02 r03 r03 r06
t37	1	r01 r02 r02 r03 r03 r03 r03 r06 r05
t38	1	r01 r02 r29
t39	1	r01 r09 r03 r10 r02 r03 r06 r05
t40	1	r01 r12 r02 r02 r03 r06 r05
t42	3	r01 r12 r02 r02 r03 r13 r03 r06 r05
t43	4	r01 r02 r03 r31 r03 r06 r05
t44	1	r01 r02 r31 r03 r06 r05
t45	1	r01 r02 r03 r31 r03 r06 **r32**
t46	1	r01 r02 r03 r13 **r33** r05
t47	1	r01 r02 r03 r03 r03 r03 r03 **r34** r06 r05
t48	1	r01 r02 r03 r03 **r31** r03 r06 r05
NT	157	

NT, non-typeable.
[a] New repeats are highlighted in bold: **r31** (AAA GAA GAC AAA GCT GAA GAT AAA GGT AGC); **r32** (AAA AAA GGC AAA GCT GCA GAC AAA GGT ATG); **r33** (AAA GAA GAC AAA GCT AAA GAC AAA GAC AAC); and **r34** (AAA GAA GAT AAA GCT AAA GAC AAA GAC AGC).

but also with other *spa* types such as t05, t06, t15 and t23 [2,21]. In this study, 34 of 41 MRSP belonged to the t02, t05, t06 or t23 types and harboured the SCC*mec* II-III cassette, indicating that the typical European clonal type is largely responsible for the dissemination of MRSP in France. The ST71–t06–SCC*mec* II-III clone, responsible for a nosocomial outbreak in Southern France [4], is not widespread since it was only identified in three dogs in this collection. Aside from the typical European clonal type, the sporadic emergence of atypical clones was observed as exemplified by the seven isolates in cluster B that were unrelated to ST71 and belonged to ST258 ($n = 3$) and to four new sequence types (ST193, ST194, ST293 and ST294).

MSSP showed a much higher genetic diversity, as suggested by other studies [6,7,21]. This is demonstrated here by the large number of different *spa* types identified, most of which were novel. The low number of *spa*-typeable isolates indicates that *spa* typing is not suited for MSSP. Whilst PFGE appears to be the most reliable method for typing MSSP, a new quick, inexpensive and robust sequence-based method is needed. Interestingly, except t41, all *spa* types identified amongst MRSP were also observed among the MSSP. This could indicate that an MSSP–MRSP conversion by acquisition of SCC*mec*, or an MRSP–MSSP reversion by the loss of SCC*mec*, might occur.

In conclusion, this study, which is the first overview of MRSP prevalence in France, demonstrates that the proportion of MRSP in clinical isolates in 2010 was 16.9% and that these opportunistic pathogens have disseminated clonally with a few local micro-evolutions.

Furthermore, these results showed that MSSP also present multiresistant profiles, including resistance to treatment options (e.g. enrofloxacin and clindamycin), and thus may emerge as a major and difficult-to-treat pathogen in the future. This increase might be due to antibiotic selection pressure or the exchange of mobile genetic elements carrying resistance determinants between MSSP and MRSP or other resistant bacteria colonising the same ecological niche.

These data highlight the need for continuous surveillance both of MSSP and MRSP as well as the development of novel therapeutic alternatives.

Funding

This work was supported by the French Food Safety Agency (Anses).

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

The authors particularly thank Jean-François Rousselot and Eric Guaguère, veterinarians in small animal practice, for their help regarding antibiotic use in dogs in France.

References

[1] van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyorala S, et al. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 2011;66:2705–14.
[2] Perreten V, Kadlec K, Schwarz S, Groenlund Andersson U, Finn M, Greko C, et al. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother 2010;65:1145–54.
[3] Osland AM, Vestby LK, Fanuelsen H, Slettemeas JS, Sunde M. Clonal diversity and biofilm-forming ability of methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 2012;67:841–8.
[4] Haenni M, Châtre P, Keck N, Franco A, Battisti A, Madec JY. Hospital-associated meticillin-resistant *Staphylococcus pseudintermedius* in a French veterinary hospital. J Global Antimicrob Resist 2013;1:225–7.
[5] 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.
[6] Gómez-Sanz E, Torres C, Lozano C, Zarazaga M. High diversity of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* lineages and toxigenic traits in healthy pet-owning household members. Underestimating normal household contact? Comp Immunol Microbiol Infect Dis 2013;36:83–94.
[7] Bannoehr J, Ben Zakour NL, Waller AS, Guardabassi L, Thoday KL, van den Broek AHM, et al. Population genetic structure of the *Staphylococcus intermedius* group: insights into *agr* diversification and the emergence of methicillin-resistant strains. J Bacteriol 2007;189:8685–92.
[8] Blaiotta G, Fusco V, Ercolini D, Pepe O, Coppola S. Diversity of *Staphylococcus* species strains based on partial *kat* (catalase) gene sequences and design of a PCR-restriction fragment length polymorphism assay for identification and differentiation of coagulase-positive species (*S. aureus*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans*). J Clin Microbiol 2010;48:192–201.
[9] Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, et al. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol 2010;48:765–9.
[10] Moodley A, Stegger M, Bagcigil AF, Baptiste KE, Loeffler A, Lloyd DH, et al. *spa* typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. J Antimicrob Chemother 2006;58:1118–23.
[11] Mick V, Domínguez MA, Tubau F, Liñares J, Pujol M, Martín R. Molecular characterization of resistance to rifampicin in an emerging hospital-associated methicillin-resistant *Staphylococcus aureus* clone ST228, Spain. BMC Microbiol 2010;10:68.
[12] Solyman SM, Black CC, Duim B, Perreten V, van Duijkeren E, Wagenaar JA, et al. Multilocus sequence typing for characterization of *Staphylococcus pseudintermedius*. J Clin Microbiol 2013;51:306–10.
[13] Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 2007;51:264–74.

[14] Ruscher C, Lübke-Becker A, Wleklinski C-G, Soba A, Wieler LH, Walther B. Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and Equidaes. Vet Microbiol 2009;136:197–201.
[15] Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach G-F, Kreienbrock L, et al. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. Vet Microbiol 2011;150:191–7.
[16] Wang Y, Yang J, Logue CM, Liu K, Cao X, Zhang W, et al. Methicillin-resistant *Staphylococcus pseudintermedius* isolated from canine pyoderma in North China. J Appl Microbiol 2012;112:623–30.
[17] Feng Y, Tian W, Lin D, Luo Q, Zhou Y, Yang T, et al. Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in pets from South China. Vet Microbiol 2012;160:517–24.
[18] Ganiere JP, Medaille C, Mangion C. Antimicrobial drug susceptibility of *Staphylococcus intermedius* clinical isolates from canine pyoderma. J Vet Med B Infect Dis Vet Public Health 2005;52:25–31.
[19] Murayama N, Nagata M, Terada Y, Okuaki M, Takemura N, Nakaminami H, et al. In vitro antiseptic susceptibilities for *Staphylococcus pseudintermedius* isolated from canine superficial pyoderma in Japan. Vet Dermatol 2013;24. 126–129.e29.
[20] Maaland M, Guardabassi L. In vitro antimicrobial activity of nitrofurantoin against *Escherichia coli* and *Staphylococcus pseudintermedius* isolated from dogs and cats. Vet Microbiol 2011;151:396–9.
[21] Ruscher C, Lubke-Becker A, Semmler T, Wleklinski CG, Paasch A, Soba A, et al. Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. Vet Microbiol 2010;144:340–6.