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Genetic characterisation of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in pets and veterinary personnel in Iran: new insights into emerging methicillin-resistant *S. pseudintermedius* (MRSP)

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ABSTRACT

Objectives: Methicillin-resistant staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), pose a threat to animal and human health worldwide. Veterinary staff and pets may play a role in the spread of resistant clones.
Methods: A total of 125 samples from veterinary staff ($n = 50$), dogs ($n = 49$) and cats ($n = 26$) were investigated. Obtained isolates were tested for the methicillin resistance gene *mecA* and were subjected to multiplex PCR to differentiate coagulase-positive species. Following *SCCmec* and *spa* typing, isolates were tested for the presence of various toxin and virulence genes and phenotypic resistance to common antimicrobials.
Results: Overall, 4 MRSA were isolated from two veterinarians and two dogs and 19 MRSP were found in eleven dogs (12 isolates) and five cats (7 isolates). The MRSA isolates possessed *sea* (2) and *eta* (3) virulence genes and the MRSP isolates possessed *sea* (6), *expA* (15), *expB* (1) and *siet* (19) genes. *SCCmec* type II and three *spa* types (t186, t1816 and t10897) were identified in the MRSA isolates. Most of the MRSP isolates belonged to SCC*mec* types II (2 isolates) and V (10 isolates); however, the remaining 7 isolates were untypeable and contained class C1 *mec*. The majority of isolates were multidrug-resistant (MDR).
Conclusion: These findings show that pets and veterinarians could be potential sources of MDR-MRSA and MDR-MRSP in Iran. Taken together, these findings warrant future investigations on the epidemiology and public-health significance of MDR-MRSA and MDR-MRSP both in veterinarians and companion animals in Iran.

1. Introduction

Staphylococcus aureus and *Staphylococcus pseudintermedius* are two common coagulase-positive staphylococci (CoPS) species frequently isolated from the skin and upper respiratory tract of humans or animals. Local infections of the skin and soft tissues as well as food poisoning are common clinical manifestations of *S. aureus* infection in humans [1]. Cats and dogs can be similarly affected by *S. aureus* and develop infections such as wound- and surgery-associated infections, pyoderma and otitis [2]. *Staphylococcus pseudintermedius* opportunistically causes frequent ear and skin infections in cats and dogs but rarely infects or colonises humans [3]. The persistency and pathogenicity of *Staphylococcus* strains in their host are attributed to several virulence factors, of which enterotoxins, exfoliative toxins and toxic shock syndrome toxin-1 significantly contribute to their pathogenesis [4].

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One of the common characteristics of CoPS is their ability to develop antimicrobial resistance. Resistance to methicillin owing to a modified penicillin-binding protein 2a (PBP2a) encoded by the *mecA* gene is a clinically important resistance phenotype amongst CoPS and, furthermore, is frequently associated with a multidrug-resistant (MDR) phenotype [3,5]. Because of limited choices of available antibiotics, infections caused by methicillin-resistant *S. aureus* (MRSA) or methicillin-resistant *S. pseudintermedius* (MRSP) are difficult to treat [2,3]. Importantly, since 2006 the global spread of highly-resistant MRSP clones has posed a challenging problem in veterinary antimicrobial therapy [6].

To broaden our knowledge regarding the epidemiology and significance of methicillin-resistant CoPS, it is essential to find major epidemiological sources in different geographic locations. The epidemiology in companion animals is of particular interest because pets are in close contact with humans and therefore resistant strains can be transmitted from these animals to humans or vice versa [2,3].

To the best of our knowledge, there is no information regarding the epidemiology and characteristics of MRSA and MRSP in pets and veterinary personnel in Iran. Thus, the aim of this study was to detect and characterise MRSA and MRSP isolates in the mentioned populations.

2. Materials and methods

2.1. Sample collection

Animals were selected on the basis of their admission to selected veterinary clinics on the day of sampling. From November 2012 to March 2013, a total of 125 samples were collected from apparently healthy humans ($n = 50$), dogs ($n = 49$) and cats ($n = 26$) without a recent history of antimicrobial use ($\geq$4 weeks). Two cat cases, misidentified as apparently healthy at the time of sampling, were also retained in the study. Samples were collected from both nostrils and the perianal area of each companion animal referred to six veterinary clinics in Tehran ($n = 4$), Karaj ($n = 1$) and Garmsar ($n = 1$). Human samples were taken from the nostrils of veterinary personnel of the aforementioned clinics, including veterinarians, technicians, veterinary students and administrative staff, with their consent.

2.2. Isolation of Staphylococcus spp

Wet sterilised swabs were rubbed against each sampling site three times, were streaked immediately onto mannitol salt agar (Merck, Darmstadt, Germany) and were incubated at 37 °C for 48–72 h. Colonies with distinct morphologies and properties were picked and were subcultured onto Columbia sheep blood agar (HiMedia, Mumbai, India). Following overnight incubation at 37 °C, suspect isolates were examined by Gram staining and catalase and oxidase tests. Gram-positive, catalase-positive, oxidase-negative cocci were considered as *Staphylococcus* spp. The isolates were further confirmed by other biochemical tests including coagulase, DNase, acetoin production and other conventional biochemical tests. Isolates were stored in brain–heart infusion broth containing 30% glycerol at −70 °C.

2.3. Screening PCR for mecA and vanA genes in coagulase-positive staphylococcal species

Total genomic DNA was extracted using a commercial DNA extraction kit for Gram-positive bacteria (CinnaGen, Tehran, Iran) and PCR was performed to detect the *mecA* gene according to the method described by Ishihara et al. [7]. Presence of the *vanA* gene encoding acquired vancomycin resistance was additionally investigated in all isolates possessing the *mecA* gene by PCR assay [8]. Vancomycin-resistant *Enterococcus faecalis* (Ferdowsi University of Mashhad, Mashhad, Iran) and sterile water were used as positive and negative controls, respectively.

2.4. Molecular identification and virulence genes of coagulase-positive staphylococcal species

Since phenotypic tests might not be accurate for identification at the species level, PCR was conducted on all *mecA*-harbouring CoPS isolates to identify the species as described by Sasaki et al. [9]. Positive and negative controls (microbial collection, University of Tehran, Tehran, Iran) were included in all PCR reactions. The presence of classical enterotoxins (*sea–see*), exfoliative toxins (*expA* and *expB* for MRSP and *eta* and *etb* for MRSA) and toxic shock syndrome toxin-1 (*tsst-1*) genes were investigated in MRSA and MRSP isolates using previously developed PCR protocols [10,11]. Detection of the *siet* gene encoding exfoliative toxin of *S. pseudintermedius* in MRSP isolates was performed as previously described by Lautz et al. [12].

2.5. Antimicrobial susceptibility testing of mecA-positive isolates

For *mecA*-positive isolates, antimicrobial susceptibility was performed by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (document VET01-A4) [13] for the following antibiotics: oxacillin (5 μg) (for *S. pseudintermedius*); cefoxitin (30 μg) (for *S. aureus*); tetracycline (30 μg); erythromycin (15 μg); gentamicin (10 μg); vancomycin (30 μg); lincomycin (2 μg); and florfenicol (30 μg).

2.6. Staphylococcal cassette chromosome mec (SCCmec) typing

SCC*mec* typing of MRSA and MRSP isolates was performed according to Kondo et al. using multiplex PCRs 1 and 2 [14]. MRSA isolates were subjected to *spa* typing and the *spa* types were assigned through the Ridom SpaServer (http://spa.ridom.de) as reported by Harmsen et al. [15].

3. Results

3.1. Genetic characterisation of mecA-positive isolates

PCR of the *mecA* gene showed positive amplification in 23 *Staphylococcus* spp. isolates, which were thus considered as methicillin-resistant and were further characterised. These isolates were identified by PCR as 4 *S. aureus* and 19 *S. pseudintermedius*. The four MRSA isolates were isolated from nostril swabs of two veterinarians and two healthy dogs. The toxin genes *sea* and *eta* were detected in two and three MRSA isolates, respectively. The four MRSA isolates harboured the SCC*mec* type II element; however, these isolates belonged to three *spa* types (t186, from a dog), t1816 (from two veterinarians) and t10897 (from a dog) (Table 1).

The 19 MRSP were isolated from 11 dogs (12 isolates) and 5 cats (7 isolates) (Table 1). Eleven and eight MRSP isolates were recovered from nostrils and the perianal area, respectively. The *sea*, *expA*, *expB* and *siet* genes were detected in 6, 15, 1 and 19 isolates, respectively. SCC*mec* types II and V were detected in 2 and 10 MRSP isolates, respectively. Interestingly, the class C *mec* complex was present in the remaining 7 MRSP isolates but their *ccr* complexes were non-typeable according to the classical scheme used in this study (Table 1) [14]. None of the *mecA*-positive isolates in the current study carried the *vanA* resistance gene or the *seb*, *sec*, *sed*, *see* or *tsst-1* genes.

Table 1
Characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strains isolated from veterinary personnel, dogs and cats in Iran.

Isolate no.	Origin	Virulence genes											*SCCmec* type	*spa* type	Resistance profile[a]
		sea	*seb*	*sec*	*sed*	*see*	*expA*	*expB*	*eta*	*etb*	*tsst-1*	*siet*			
MRSA1	Human	–	–	–	–	–	n.a	n.a	+	–	–	n.t	II	t1816	TET, ERY
MRSA2	Human	+	–	–	–	–	n.a	n.a	+	–	–	n.t	II	t1816	TET, ERY
MRSA3	Dog	+	–	–	–	–	n.a	n.a	+	–	–	n.t	II	t186	GEN, TET, LIN, ERY
MRSA4	Dog	–	–	–	–	–	n.a	n.a	–	–	–	n.t	II	t10897	GEN, LIN, FLR, ERY
MRSP1	Cat	–	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP2	Cat	–	–	–	–	–	+	–	n.a	n.a	–	+	NT (class C)	n.a	TET, LIN, ERY
MRSP3	Dog	–	–	–	–	–	–	–	n.a	n.a	–	+	NT (class C)	n.a	GEN, TET, LIN, ERY
MRSP4	Dog	–	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP5	Cat	+	–	–	–	–	–	–	n.a	n.a	–	+	II	n.a	GEN, TET, LIN, ERY
MRSP6	Dog	–	–	–	–	–	+	–	n.a	n.a	–	+	NT (class C)	n.a	GEN, TET
MRSP7	Dog	–	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP8	Cat	–	–	–	–	–	–	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP9	Dog	–	–	–	–	–	+	–	n.a	n.a	–	+	NT (class C)	n.a	GEN, FLR
MRSP10	Cat	–	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP11	Dog	+	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP12	Dog	–	–	–	–	–	+	+	n.a	n.a	–	+	NT (class C)	n.a	TET
MRSP13	Dog	+	–	–	–	–	–	–	n.a	n.a	–	+	V	n.a	ERY
MRSP14	Dog	+	–	–	–	–	+	–	n.a	n.a	–	+	NT (class C)	n.a	GEN, TET, LIN, ERY
MRSP15	Cat	+	–	–	–	–	+	–	n.a	n.a	–	+	II	n.a	GEN, TET, LIN, ERY
MRSP16	Dog	–	–	–	–	–	+	–	n.a	n.a	–	+	NT (class C)	n.a	TET, ERY
MRSP17	Dog	–	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP18	Cat	–	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY
MRSP19	Dog	+	–	–	–	–	+	–	n.a	n.a	–	+	V	n.a	GEN, TET, LIN, ERY

SCC*mec*, staphylococcal cassette chromosome *mec*; n.a, not applicable; n.t, not tested; NT, non-typeable; TET, tetracycline; ERY, erythromycin; GEN, gentamicin; LIN, lincomycin; FLR, florfenicol.
[a] For simplicity, resistance to β-lactams was excluded from the profiles.

3.2. Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolates

In addition to oxacillin/cefoxitin resistance, other resistance phenotypes were observed among the MRSA and MRSP isolates, including to gentamicin (2/4 MRSA; 15/19 MRSP), tetracycline (3/4; 17/19), lincomycin (2/4; 14/19) and erythromycin (4/4; 16/19) (Table 1). Overall, three resistance profiles were observed in the MRSA isolates, whilst seven profiles were determined in MRSP isolates with one prevailing. The predominant MRSP resistance profile ($n = 13$ isolates) showed resistance to gentamicin, tetracycline, lincomycin and erythromycin (Table 1).

4. Discussion

The fact that MRSA and MRSP can colonise small animals and veterinary staff necessitates continuous monitoring for these pathogens in order to prevent and control related infections. In the present study, the occurrence of MRSP in dogs (22.4%; 11/49) and cats (19.2%; 5/26) was considerably high, similar to previous reports in Thailand (45% of apparently healthy dogs) and Japan (30% and 66% of dogs with skin infection) [3,16]. It should be noted that the occurrence of methicillin-resistant staphylococci in the present study might be underestimated because a non-selective approach was used for primary isolation. Nevertheless, the prevalence of MRSP in the majority of previous studies ranged from 0% to 6.2% [3,17]. Rota et al. have stated that improper use of antibiotics can cause selection of and longer colonisation by MRSP strains in healthy dogs [18]. Although there is no detailed report available on antibiotic consumption in companion animals in Iran, use of antibacterial agents is considered to be high both in humans and food-producing animals [19,20]. The trend and attitudes towards antibiotic use in Iran may explain, at least in part, the high frequency of MRSP isolation in companion animals in this study since antibiotic treatment is a risk factor for MRSP colonisation [3].

None of the MRSP isolates contained the hybrid element SCC*mec* type II–III associated with the major MRSP lineage in Europe as well in Japan and North China [21–23]. Ten MRSP strains harboured SCC*mec* type V, commonly found in major MRSP clones of North America, South China, Japan, Thailand and South Korea [7,16,17,21,24–26]. SCC*mec* type II was detected in two MRSP isolates, which is rare and formerly identified in Japanese MRSP strains [26]. SCC*mec* was non-typeable in seven MRSP isolates, which contained a class C1 *mec* type but no *ccr* genes [14]; this may reflect the presence of a pseudo-SCC*mec* element. Other studies have also reported the occurrence of such non-typeable SCC*mec* in MRSP as well as in other methicillin-resistant staphylococci species such as *S. aureus*, *Staphylococcus hyicus, Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates from infections in humans, livestock and companion animals [27–29].

Concerns regarding the importance of MRSA in Iran have expanded in recent years. A recent meta-analysis in Iran estimated the overall frequency of MRSA to be 43% in humans [30]. In the present study, two human MRSA isolates were isolated from two veterinarians in the same clinic exhibiting identical SCC*mec* (type II) and *spa* types (t1816) and identical antimicrobial resistance profiles. This finding suggests person-to-person transmissibility and colonisation of resistant strains in healthcare personnel and further possible spread to the community. The observed MRSA frequency in dogs (4.1%) and cats (0%) is in accordance with findings of numerous investigations reporting a low occurrence of MRSA in dogs and cats ranging from 0% to 1.5% [31], and similar to that reported for a healthy human population [32]. A recent comprehensive study reported 4% MRSA in animals and veterinarians in Australia [33], which is comparable with the present report. It should be noted that the overall frequency of MRSA was reported at much higher rates in humans in Iran as mentioned previously [30].

One of the two MRSA isolated from veterinarians was enterotoxigenic (containing the *sea* gene). Similarly, enterotoxigenic MRSA have been found in Swiss veterinarians [34]. Previous information on the presence of exfoliative toxin genes in *S. aureus* isolated from veterinarians and pets appears to be scarce. Interestingly, in the present study, one dog and both veterinarian

MRSA isolates contained the *eta* gene. In comparison, one study in the UK also documented two methicillin-susceptible *S. aureus* strains carrying the *eta* gene [35]. Most of the MRSP isolates from dogs and cats in the current study carried the *expA* gene, whilst one dog isolate harboured both *expA* and *expB*.

No hospital-associated MRSA was found in this study. Indeed, as mentioned above, the four MRSA isolates were SCC*mec* type II, but previous investigations in Iran revealed that SCC*mec* types III and IV are the common MRSA types from hospitalised individuals [36,37]. Based on the Ridom SpaServer database (http://spa.ridom.de), the observed *spa* types t186 and t10897 (detected in two dogs) are infrequent and only *spa* type t1816 has been previously reported in Iran. With a low frequency, t1816 has been identified in several countries, whilst t10897 has so far only been reported from Norway (based on the available *spa* types database). Considering SCC*mec* and *spa* types of the isolated strains, they were not related to hospital-associated MRSA strains.

MDR-MRSA and MDR-MRSP isolated from companion animals are emerging worldwide, and high resistance to tetracycline and erythromycin, similar to the current study, has been frequently reported [16,17,21,24,38]. Therefore, cats and dogs should be considered as potential reservoirs of MDR-MRSA and MDR-MRSP strains, which seriously threaten animal and public health by limiting the choice of available antibiotics to treat related infections. In dogs carrying MRSP strains, consumption of antibiotics to which MRSP are resistant can extend their colonisation period [39]. The susceptibility of MDR-MRSA and MDR-MRSP to florfenicol makes it a suitable antibiotic agent to treat related infections. Recently, Maaland et al. have documented the susceptibility of MRSP to florfenicol and propose it for use against MRSP after further pharmacological evaluation [40].

In conclusion, the results of this study revealed colonisation of healthy veterinarians, dogs and cats with MDR-MRSA and MDR-MRSP possessing important virulence genes for the first time in Iran. Considering the sample size of this study, future studies with a larger number of samples are required to clarify more details on the epidemiology and public-health significance of MRSA and MRSP in Iran. Moreover, clonal analysis of MRSA/MRSP strains by multilocus sequence typing (MLST) may help to detect the possible emergence of new genetic lineages among the human or animal populations in Iran [16].

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Competing interests

None declared.

Ethical approval

Approved by the Research Committee of the University of Tehran (Tehran, Iran) [no. 10/3/1391].

References

[1] Xu SX, McCormick JK. Staphylococcal superantigens in colonization and disease. Front Cell Infect Microbiol 2012;52:1–11.
[2] Weese JS. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J 2010;51:233–44.
[3] van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyörälä S, et al. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 2011;66:2705–14.
[4] Plano LEW. *Staphylococcus aureus* exfoliative toxins: how they cause disease. J Invest Dermatol 2004;122:1070–7.
[5] Stryjewski ME, Corey GR. Methicillin-resistant *Staphylococcus aureus*: an evolving pathogen. Clin Infect Dis 2014;58:10–9.
[6] Guardabassi L, Larsen J, Weese JS, Butaye P, Battisti A, Kluytmans J, et al. Public health impact and antimicrobial selection of meticillin-resistant staphylococci in animals. J Glob Antimicrob Resist 2013;1:55–62.
[7] Ishihara K, Shimokubo N, Sakagami A, Ueno H, Muramatsu Y, Kadosawa T, et al. Occurrence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in an academic veterinary hospital. Appl Environ Microbiol 2010;76:5165–74.
[8] Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother 1993;37:2311–7.
[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] Mehrotra M, Wang G, Johnson WM, Multiplex PCR. for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000;38:1032–5.
[11] Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou Elnaga Y, et al. Sharing more than friendship: nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS One 2012;7:35197.
[12] Lautz S, Kanbar T, Alber J, Lämmler C, Weiss R, Prenger-Berninghoff E, et al. Dissemination of the gene encoding exfoliative toxin of *Staphylococcus intermedius* among strains isolated from dogs during routine microbiological diagnostics. J Vet Med B Infect Dis Vet Public Health 2006;53:434–8.
[13] Clinical Laboratory Standard Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard—fourth edition. Document VET01-A4. Wayne, PA: CLSI; 2013.
[14] 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.
[15] Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 2003;41:5428–42.
[16] Chanchaithong P, Perreten V, Schwendener S, Tribuddharat C, Chongthaleong A, Niyomtham W, et al. Strain typing and antimicrobial susceptibility of methicillin-resistant coagulase-positive staphylococcal species in dogs and people associated with dogs in Thailand. J Appl Microbiol 2014;117:572–86.
[17] Davis JA, Jackson CR, Fedorka-Cray PJ, Barrett JB, Brousse JH, Gustafson J, et al. Carriage of methicillin-resistant staphylococci by healthy companion animals in the US. Lett Appl Microbiol 2014;59:1–8.
[18] Rota A, Milani C, Corrò M, Drigo I, Börjesson S. Misuse of antimicrobials and selection of methicillin-resistant *Staphylococcus pseudintermedius* strains in breeding kennels: genetic characterization of bacteria after a two-year interval. Reprod Domest Anim 2013;48:1–6.
[19] Abdollahiasl A, Kebriaeezadeh A, Nikfar S, Farshchi A, Ghiasi G, Abdollahi M. Patterns of antibiotic consumption in Iran during 2000–2009. Int J Antimicrob Agents 2011;37:489–90.
[20] Aalipour F, Mirlohi M, Jalali M. Determination of antibiotic consumption index for animal originated foods produced in animal husbandry in Iran, 2010. J Environ Health Sci Eng 2014;12:1–7.
[21] Perreten V, Kadlec K, Schwarz S, Grönlund 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.
[22] Ishihara K, Koizumi A, Saito M, Muramatsu Y, Tamura Y. Detection of methicillin-resistant *Staphylococcus pseudintermedius* ST169 and novel ST354 SCC*mec* II–III isolates related to the worldwide ST71 clone. Epidemiol Infect 2015;144:434–42.
[23] 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.
[24] 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.
[25] Youn JH, Koo HC, Ahn KJ, Lim SK, Park YH. Determination of staphylococcal exotoxins, SCC*mec* types, and genetic relatedness of *Staphylococcus intermedius* group isolates from veterinary staff, companion animals, and hospital environments in Korea. J Vet Sci 2011;12:221–6.
[26] Bardiau M, Yamazaki K, Ote I, Misawa N, Mainil JG. Characterization of methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs and cats. Microbiol Immunol 2013;57:496–501.
[27] Kang JH, Chung TH, Hwang CY. Clonal distribution of methicillin-resistant *Staphylococcus pseudintermedius* isolates from skin infection of dogs in Korea. Vet Microbiol 2017;210:32–7.

[28] Park J, Friendship RM, Weese JS, Poljak Z, Dewey CE. An investigation of resistance to (-lactam antimicrobials among staphylococci isolated from pigs with exudative epidermitis. BMC Vet Res 2013;9:211.
[29] McManus BA, Coleman DC, Deasy EC, Brennan GI, O'Connell B, Monecke S, et al. Comparative genotypes, staphylococcal cassette chromosome *mec* (*SCCmec*) genes and antimicrobial resistance amongst *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates from infections in humans and companion animals. PLoS One 2015;10:1–18.
[30] Dadashi M, Nasiri MJ, Fallah F, Owlia P, Hajikhani B, Emaneini M, et al. Methicillin resistant *Staphylococcus* in Iran: a systematic review and meta-analysis. J Glob Antimicrob Resist 2018;12:96–103.
[31] Loeffler A, Pfeiffer DU, Lindsay JA, Soares Magalhães RJ, Lloyd DH. Prevalence of and risk factors for MRSA carriage in companion animals: a survey of dogs, cats and horses. Epidemiol Infect 2011;139:1019–28.
[32] Wulf M, van Nes A, Eikelenboom-Boskamp A, de Vries J, Melchers W, Klaassen C, et al. Methicillin-resistant *Staphylococcus aureus* in veterinary doctors and students, the Netherlands. Emerg Infect Dis 2006;12:1939–41.
[33] Worthing KA, Abraham S, Pang S, Coombs GW, Saputra S, Jordan D, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from Australian animals and veterinarians. Microb Drug Resist 2018;24:203–12.
[34] Wettstein Rosenkranz K, Rothenanger E, Brodard I, Collaud A, Overesch G, Bigler B, et al. Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among Swiss veterinary health care providers: detection of livestock- and healthcare-associated clones. Schweiz Arch Tierheilkd 2014;156:317–25.
[35] Wedley AL, Dawson S, Maddox TW, Coyne KP, Pinchbeck GL, Clegg P, et al. Carriage of *Staphylococcus* species in the veterinary visiting dog population in mainland UK: molecular characterisation of resistance and virulence. Vet Microbiol 2014;170:81–8.
[36] Japoni-Nejad A, Rezazadeh M, Kazemian H, Fardmousavi N, van Belkum A, Ghaznavi-Rad E. Molecular characterization of the first community-acquired methicillin-resistant *Staphylococcus aureus* strains from Central Iran. Int J Infect Dis 2013;17:949–54.
[37] Mohammadi S, Sekawi Z, Monjezi A, Maleki MH, Soroush S, Sadeghifard N, et al. Emergence of SCC*mec* type III with variable antimicrobial resistance profiles and *spa* types among methicillin-resistant *Staphylococcus aureus* isolated from healthcare- and community-acquired infections in the west of Iran. Int J Infect Dis 2014;25:152–8.
[38] Garbacz K, Żarnowska S, Piechowicz L, Haras K. Pathogenicity potential of *Staphylococcus pseudintermedius* strains isolated from canine carriers and from dogs with infection signs. Virulence 2013;4:255–9.
[39] Windahl U, Reimegård E, Holst BS, Egenvall A, Fernström L, Fredriksson M, et al. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs—a longitudinal study. BMC Vet Res 2012;8:34.
[40] Maaland MG, Mo SS, Schwarz S, Guardabassi L. In vitro assessment of chloramphenicol and florfenicol as second-line antimicrobial agents in dogs. J Vet Pharmacol Ther 2015;38:443–50.