

Original Article

Determination of staphylococcal exotoxins, SCCmec types, and genetic relatedness of *Staphylococcus intermedius* group isolates from veterinary staff, companion animals, and hospital environments in Korea

Jung-Ho Youn¹, Hye Cheong Koo^{1,*}, Kuk Ju Ahn¹, Suk-Kyung Lim², Yong Ho Park^{1,*}

¹Department of Microbiology, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul 151-742, Korea

²National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang 430-856, Korea

The *Staphylococcus* (*S.*) *intermedius* group (SIG) has been a main research subject in recent years. *S. pseudintermedius* causes pyoderma and otitis in companion animals as well as foodborne diseases. To prevent SIG-associated infection and disease outbreaks, identification of both staphylococcal exotoxins and staphylococcal cassette chromosome *mec* (SCCmec) types among SIG isolates may be helpful. In this study, it was found that a single isolate (one out of 178 SIG isolates examined) harbored the canine enterotoxin SEC gene. However, the *S. intermedius* exfoliative toxin gene was found in 166 SIG isolates although the *S. aureus*-derived exfoliative toxin genes, such as *eta*, *etb* and *etd*, were not detected. SCCmec typing resulted in classifying one isolate as SCCmec type IV, 41 isolates as type V (including three *S. intermedius* isolates), and 10 isolates as non-classifiable. Genetic relatedness of all *S. pseudintermedius* isolates recovered from veterinary staff, companion animals, and hospital environments was determined by pulsed-field gel electrophoresis. Strains having the same band patterns were detected in *S. pseudintermedius* isolates collected at 13 and 18 months, suggesting possible colonization and/or expansion of a specific *S. pseudintermedius* strain in a veterinary hospital.

Keywords: methicillin-resistant *Staphylococcus intermedius* group, *Staphylococcus intermedius* group, *Staphylococcus pseudintermedius*, toxin

Introduction

Staphylococcus (*S.*) *intermedius* and *S. pseudintermedius* are both *Staphylococcus intermedius* group (SIG) members.

These microorganisms are opportunistic pathogens that cause skin and nosocomial infections in dogs and cats [32]. *S. pseudintermedius* is the predominant SIG species in Korea and until recently was misclassified as *S. intermedius* [30,33]. These pathogens rarely cause human disease although they do have zoonotic potential [5,6,26]. Increased identification of methicillin-resistant SIG (MRSIG) isolates has emphasized the importance of public health precautions for veterinary staff and pet owners [7,11,26].

Many current studies have evaluated methicillin-resistant characteristics and the staphylococcal cassette chromosome *mec* (SCCmec) of SIG isolates, particularly *S. pseudintermedius*. Although type I to type VIII SCCmec types have been identified, there is no significant studies have been conducted to examine SCCmec types isolated from veterinary hospitals located nationwide in Korea. SCCmec types may produce staphylococcal exotoxins including staphylococcal enterotoxins (SEs), exfoliative toxins (ETs) including *S. intermedius* exfoliative toxin (SIET), and toxic shock syndrome toxin 1 (TSST 1); these toxins are associated with atopic dermatitis, mastitis, food poisoning, canine pyoderma, and chronic otitis [1,3,10,14,29]. SIET (encoded by the *siet* gene), first described by Terauchi *et al.* [27], plays a potential role in the pathogenesis of canine pyoderma and chronic otitis [31]. There may also be a greater chance of SIG isolates to colonization and/or expand in veterinary staff, companion animals, and hospital environments. Therefore, this study is focused on the genetic identification of staphylococcal exotoxins, SCCmec types, and genetic relatedness by pulsed-field gel electrophoresis (PFGE) of the SIG isolates in Korea.

Materials and Methods

Samples of *S. pseudintermedius* (n = 167) and *S. intermedius*

*Corresponding authors

Tel: +82-2-880-1257; Fax: +82-2-871-7524

E-mail: koohj@snu.ac.kr, yhp@snu.ac.kr

($n = 11$) were isolated; their identity was confirmed by Gram-staining and biochemical testing such as coagulase, DNase production and hydrolysis [33]. Polymerase chain reaction (PCR) was carried out with primers targeting *S. intermedius* nuclease and 16S rRNA genes [2]. To differentiate *S. pseudintermedius* from *S. intermedius* and *S. delphini*, PCR-restriction fragment length polymorphism analysis was performed using *pta* gene-specific primers (*pta*_{f1}: 5'-AAA GAC AAA CTT TCA GGT AA-3', and *pta*_{r1}: 5'-GCA TAA ACA AGC ATT GTA CCG-3'), and the restriction enzyme *Mbo*I (New England Biolabs, USA) [3]. Samples were collected from eight veterinary hospitals from four different regions in Korea between November 2006 and January 2010 as previously described [33]. PCR analysis to detect the methicillin-resistance gene (*mecA*) was performed with gene-specific primers as previously described [32]. PCR was also performed to amplify genes encoding SEA (*sea*), SEB (*seb*), SEC (*sec*), SED (*sed*), SEE (*see*), SEG (*seg*), SEH (*seh*), SEI (*sei*), TSST 1 (*tsst-1*) and ETs (*eta*, *etb*, *etd*, and *siet*) using primers and PCR conditions used in other previous studies [12,16,19,20, 24,25]. FRI913, FRI 361, FRI 472, FRI 569, MNHOCH, and RN4220 strains were used as either positive or negative controls for superantigen genes. A superantigen gene obtained in this study was further analyzed by DNA sequencing using the Vector NTI Align X program (Invitrogen, USA).

All MRSIG isolates including 49 methicillin-resistant *S. pseudintermedius* (MRSP) and three methicillin-resistant *S. intermedius* (MRSI) were subsequently classified as SCC*mec* type I to VIII by either single or multiplex PCR assays using protocols in other previous studies including primers and PCR conditions [4,9,13,15,17,18]. Genomic

DNA plug kits (Bio-Rad, USA) with *Sma*I (New England Biolabs, USA) digestion were used for the PFGE analysis of 39 *S. pseudintermedius* isolates from a private referral veterinary hospital collected at different times (eight isolates from November 2006, 24 isolates from April 2008, four isolates from June 2009, and three isolates from October 2009) and six *S. intermedius* isolates (all collected during April 2008). PFGE was performed according to protocol used in previous study [8] and the manufacture's instruction (Bio-Rad, USA) except for an initial pulse time of 5 sec and final time of 40 sec for 21 h [8].

Results

Analysis of the SIG isolates ($n = 178$) for SEs and TSST 1 genes showed that only a single *S. pseudintermedius* isolate from a veterinary staff member (H1-23) harbored the enterotoxin C (*sec*) gene (Fig. 1). Subsequent DNA sequencing analysis (Fig. 2) revealed that the amplified *sec* gene the canine type C SE gene (*sec*_{canine}). A total of 166 out of 178 SIG isolates (155 *S. pseudintermedius* and 11 *S. intermedius*) isolated from veterinary staff members ($n = 38$, 95%), companion animals ($n = 107$, 93%), and veterinary hospitals ($n = 21$, 91.3%) harbored *siet* originally detected in *S. intermedius*. However, other ET genes such as *eta*, *etb*, and *etd*, known to originate from *S. aureus*, were not identified.

SCC*mec* typing of the 49 MRSP isolates identified 38 type V isolates, one type IV isolate, and 10 non-identifiable isolates. Three MRSI isolates were characterized as type V isolates (Table 1). PFGE analysis showed that SIG isolates from a private referral animal hospital (collected between 2006 and 2009) recovered from veterinary staff, com-

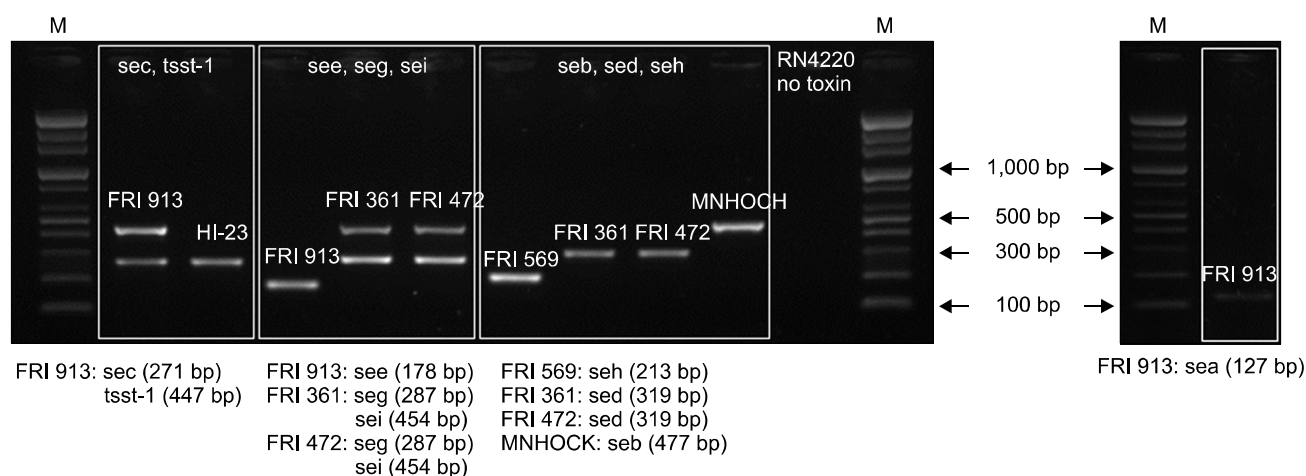


Fig. 1. Staphylococcal enterotoxins A (*sea*), B (*seb*), C (*sec*), D (*sed*), E (*see*), G (*seg*), H (*seh*), and I (*sei*), and the toxic shock syndrome toxin 1 (*tsst-1*) were detected by single or multiplex polymerase chain reaction (PCR) in 178 SIG isolates collected from veterinary staff, companion animals, and veterinary hospital environments in Korea. Genomic DNA from FRI913, FRI 361, FRI 472, FRI 569, MNHOCH, and RN4220 were used as controls. Multiplex PCR for *sec*, *tsst-1*/*see*, *seg*, *sei*/*seb*, *sed*, and *seh* and uniplex PCR for *sea* were performed. Only a single isolate from a veterinary staff (H1-23, hand) was positive for *sec*.



Fig. 2. The PCR-amplified *sec* gene sequence from the *Staphylococcus* (*S.*) *pseudintermedius* isolate H1-23 collected from a veterinary staff member was aligned with that of *sec* genes encoding *S. intermedius* SEC_{canine} from the *S. intermedius* gene. Nucleotide sequences with GenBank numbers U91526, X05815, DQ192646, and X51661 were used for the alignment analysis of *sec*_{canine}, *sec*1, *sec*2, and *sec*3, respectively. Alignment was performed using the Vector NTI Align X program.

Table 1. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of *Staphylococcus intermedius* group (SIG) isolates from Korean veterinary hospitals (veterinary staff, companion animal, and the veterinary hospital environment)

Group/SCC <i>mec</i> type	I	II	III	IV	V	VI	VII	VIII	NT*
Veterinary staff	0	0	0	0	9	0	0	0	5
Companion animal	0	0	0	0	26 [†]	0	0	0	3
Veterinary hospital environment	0	0	0	1	6	0	0	0	2
Total	0	0	0	1	41	0	0	0	10

*NT: non-typeable. [†]Included 3 *Staphylococcus* (*S.*) *intermedius* isolates and the other SIG isolates were *S. pseudintermedius*.

panion animals, and the environment had the same band patterns (Fig. 3). Six *S. pseudintermedius* isolates (two from companion animals collected in September 2006, three from veterinary staff members, and one from companion animals collected in April 2008) and two *S. pseudintermedius* isolates (one from a veterinary hospital environment isolated in April 2008, and one from a companion animal isolated in June 2009) showed the same band patterns (Fig. 3).

Discussion

Since SIG is closely related to *S. aureus*, studies have been performed to determine whether SIG has adapted the *eta*, *etb* and *etd* toxins from *S. aureus* by PCR test with specific primers [16,24,25]. None of these toxins were detected. However, 166 out of 178 (93.3%) SIG isolates harbored the *siet* toxin originating from *S. intermedius* [27]. This result and those of other studies [22,31] imply that the majority of SIG isolates harbor the *siet* gene.

Although the *siet* gene was present in 93.3% of the SIG isolates (from 108 dogs and two cats) in this study, only 14 dogs had a history of various skin disease including allergy and prolonged inflammation lesion in skin ($n = 13$) or otitis ($n = 1$) (data not shown). Therefore, other factors such as the general health of an animal and existence of other SIG virulence factors may play an important role in outbreaks of various kinds of skin disease or otitis.

The *sec* gene was detected in a single isolate in the present study, which was identified as *sec*_{canine} by DNA sequencing. However, this isolate was isolated from a veterinary staff member, and no additional SEC_{canine} isolates were identified in the veterinary hospital where this individual worked. This suggested that the isolate might be transmitted from an in- or outgoing companion animal with which the veterinary staff was in contact. The low incidence of toxins in this study could be secondary to the small number of isolates collected from companion animals that a history of skin disease or otitis. Only 11.3% of *S. pseudintermedius* in a previous study had exotoxins

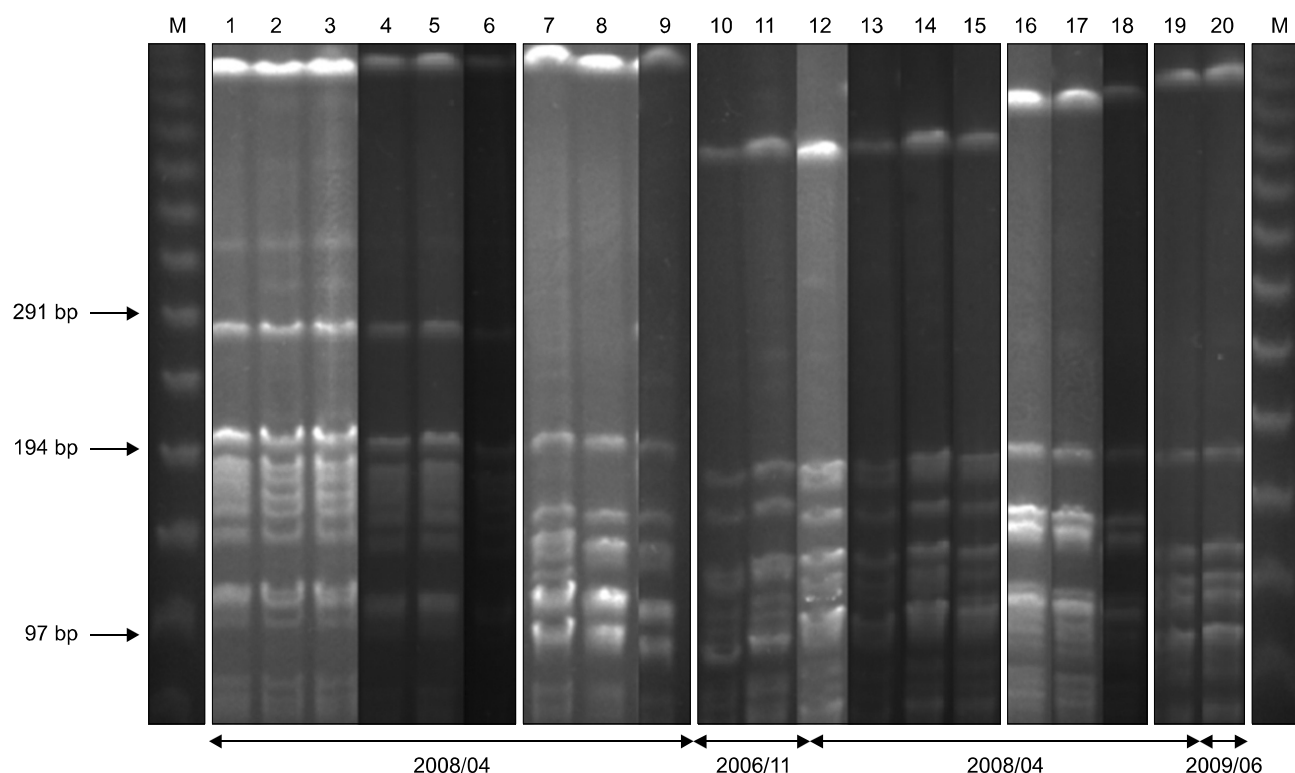


Fig. 3. Comparison of *S. intermedius* group isolates from the H veterinary hospital between September 2006 and October 2009 using pulsed-field gel electrophoresis (PFGE) to detect identical PFGE patterns between isolates from veterinary staff, companion animals, and veterinary hospital environments collected on the same and different sampling dates. Lane M: Lambda Ladder PFG Marker (50 ~ 1,000 kb); Lanes 1 ~ 3 (SI) from single CA#1 (dog: anus, nasal mucosa, skin); Lane 4 (SI), VS#1 (nasal mucosa); Lanes 5 ~ 6 (SI) from single VS#2 (nasal mucosa, hand); Lanes 7 ~ 8 (SP) from a single CA#3 (dog: ear canal, ear canal); Lane 9 (SP), VS#3 (nasal mucosa); Lanes 10 ~ 11 (SP) from a single CA#4 (dog: anus, nasal mucosa); Lane 12 (SP), CA#5 (anus); Lane 13 (SP) VS#4 (nasal mucosa); Lane 14 (SP) VS#5 (nasal mucosa); Lane 15 (SP) VS#6 (nasal mucosa); Lane 16 (SP) CA#6 (anus); Lane 17 (SP) VS#7 (nasal mucosa); Lane 18 (SP) VS#8 (nasal mucosa); Lane 19 (SP) VHE#1 (floor); Lane 20 (SP) CA#7 (anus). CA: companion animal, VS: veterinary staff, VHE: veterinary hospital environment, SI: *Staphylococcus intermedius*, SP: *Staphylococcus pseudintermedius*.

although all samples were taken from patients diagnosed with pyoderma or chronic otitis and referred to a veterinary teaching hospital [30]. This result suggested that SE toxins may be associated with pyoderma and chronic otitis.

A total of 52 out of 178 SIG isolates harbored the *mecA* gene. Although the majority of SCCmec types were type V (78.9%), one isolate was type IV. In a previous study [10], 23 isolates (85.2%), three isolates (11.1%) and one isolate (3.7%) from veterinarians, staff, students, companion animals and environment in the veterinary hospitals were determined to be an MRSP hybrid SCCmec type I~II, type V, or non-identifiable, respectively. A previous European and North American study [21] identified 75 hybrid SCCmec type II~III isolates (72.8%), two type III isolates (1.9%), six type IV isolates (5.8%), 14 type V isolates (13.6%), four type VII isolates (3.9%), and two non-identifiable isolates (1.9%) from diseased and healthy dogs in veterinary diagnostic laboratories of different countries. This demonstrated that the majority of MRSIG isolates in Korea harbor the SCCmec type V whereas the

hybrid type II~III is the main SCCmec type found in veterinary hospitals in Japan, Europe, and North America.

In the present study, PFGE analysis of the 39 *S. pseudintermedius* and six *S. intermedius* isolates from a private referral veterinary hospital (collected during November 2006, April 2008, June 2009, and October 2009) showed that 20 isolates (lines 1 ~ 6, lines 7 ~ 9, lines 10 ~ 15, lines 16 ~ 18, and lines 19 ~ 20) had the same band patterns. Moreover, some isolates obtained on different sampling dates showed the same band patterns (six isolates from lines 12 ~ 17, and two isolates from lines 21 ~ 22). These results suggest potential contamination or expansion of *S. pseudintermedius* and *S. intermedius* isolates among veterinary staff, companion animals, and veterinary hospital environments, and colonization by these specific strains for more than 13 or 18 months in the same hospital.

In conclusion, *eta*, *etb*, *etd* genes were not detected but *siet* toxin was found in 166 isolates in the current study. PFGE analysis results of isolated from H animal hospital

showed that *S. pseudintermedius* isolates collected in over a period of 13 and 18 months from veterinary staff, companion animals, and the hospital environment had the same band patterns. *S. pseudintermedius* infections in humans [5,26,28], spread of MRSP populations [6,23,29], and association of SIG with canine pyoderma and chronic otitis [3,14,29] has been previously reported. Therefore, SIG, especially MRSIG, may have significant clinical implications for companion animals with skin infections or chronic otitis that is of concern for veterinary staff, companion animal owners, and healthy companion animals.

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References

1. Balaban N, Rasooly A. Staphylococcal enterotoxins. *Int J Food Microbiol* 2000, **61**, 1-10.
2. Bannoehr J, Franco A, Iurescia M, Battisti A, Fitzgerald JR. Molecular diagnostic identification of *Staphylococcus pseudintermedius*. *J Clin Microbiol* 2009, **47**, 469-471.
3. Baron F, Cochet MF, Pellerin JL, Ben Zakour N, Lebon A, Navarro A, Proudly I, Le Loir Y, Gautier M. Development of a PCR test to differentiate between *Staphylococcus aureus* and *Staphylococcus intermedius*. *J Food Prot* 2004, **67**, 2302-2305.
4. Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I-V. *Clin Microbiol Infect* 2007, **13**, 725-727.
5. Chuang CY, Yang YL, Hsueh PR, Lee PI. Catheter-related bacteremia caused by *Staphylococcus pseudintermedius* refractory to antibiotic-lock therapy in a hemophilic child with dog exposure. *J Clin Microbiol* 2010, **48**, 1497-1498.
6. 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.
7. Futagawa-Saito K, Makino S, Sunaga F, Kato Y, Sakurai-Komada N, Ba-Thein W, Fukuyasu T. Identification of first exfoliative toxin in *Staphylococcus pseudintermedius*. *FEMS Microbiol Lett* 2009, **301**, 176-180.
8. Herschleb J, Ananiev G, Schwartz DC. Pulsed-field gel electrophoresis. *Nat Protoc* 2007, **2**, 677-684.
9. Higuchi W, Takano T, Teng LJ, Yamamoto T. Structure and specific detection of staphylococcal cassette chromosome *mec* type VII. *Biochem Biophys Res Commun* 2008, **377**, 752-756.
10. Ishihara K, Shimokubo N, Sakagami A, Ueno H, Muramatsu Y, Kadosawa T, Yanagisawa C, Hanaki H, Nakajima C, Suzuki Y, Tamura Y. Occurrence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in an academic veterinary hospital. *Appl Environ Microbiol* 2010, **76**, 5165-5174.
11. Kadlec K, Schwarz S, Perreten V, 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. Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. *J Antimicrob Chemother* 2010, **65**, 1826-1828.
12. Lautz S, Kanbar T, Alber J, Lämmler C, Weiss R, Prenger-Berninghoff E, Zschöck M. 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**, 434-438.
13. Lim SK, Nam HM, Park HJ, Lee HS, Choi MJ, Jung SC, Lee JY, Kim YC, Song SW, Wee SH. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* in raw meat in Korea. *J Microbiol Biotechnol* 2010, **20**, 775-778.
14. 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**, 412-421.
15. McClure JA, Conly JM, Elsayed S, Zhang K. Multiplex PCR assay to facilitate identification of the recently described staphylococcal cassette chromosome *mec* type VIII. *Mol Cell Probes* 2010, **24**, 229-232.
16. Nakaminami H, Noguchi N, Ikeda M, Hasui M, Sato M, Yamamoto S, Yoshida T, Asano T, Senoue M, Sasatsu M. Molecular epidemiology and antimicrobial susceptibilities of 273 exfoliative toxin-encoding-gene-positive *Staphylococcus aureus* isolates from patients with impetigo in Japan. *J Med Microbiol* 2008, **57**, 1251-1258.
17. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, Coombs GW, Pearman JW, Tenover FC, Kapi M, Tiensasitorn C, Ito T, Hiramatsu K. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002, **40**, 4289-4294.
18. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*.

- Antimicrob Agents Chemother 2002, **46**, 2155-2161.
19. **Omoe K, Ishikawa M, Shimoda Y, Hu DL, Ueda S, Shinagawa K.** Detection of *seg*, *seh*, and *sei* genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring *seg*, *seh*, or *sei* genes. J Clin Microbiol 2002, **40**, 857-862.
 20. **Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K.** Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. FEMS Microbiol Lett 2005, **246**, 191-198.
 21. **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**, 1145-1154.
 22. **Ruscher C, Lübke-Becker A, Semmler T, Wleklinski CG, Paasch A, Šoba A, Stamm I, Kopp P, Wieler LH, Walther B.** Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. Vet Microbiol 2010, **144**, 340-346.
 23. **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**, 1118-1125.
 24. **Sato H, Matsumori Y, Tanabe T, Saito H, Shimizu A, Kawano J.** A new type of staphylococcal exfoliative toxin from a *Staphylococcus aureus* strain isolated from a horse with phlegmon. Infect Immun 1994, **62**, 3780-3785.
 25. **Sila J, Sauer P, Kolar M.** Comparison of the prevalence of genes coding for enterotoxins, exfoliatins, panton-valentine leukocidin and *tsst-1* between methicillin-resistant and methicillin-susceptible isolates of *Staphylococcus aureus* at the university hospital in Olomouc. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2009, **153**, 215-218.
 26. **Stegmann R, Burnens A, Maranta CA, Perreten V.** Human infection associated with methicillin-resistant *Staphylococcus pseudintermedius* ST71. J Antimicrob Chemother 2010, **65**, 2047-2048.
 27. **Terauchi R, Sato H, Hasegawa T, Yamaguchi T, Aizawa C, Maehara N.** Isolation of exfoliative toxin from *Staphylococcus intermedius* and its local toxicity in dogs. Vet Microbiol 2003, **94**, 19-29.
 28. **Van Hoovels L, Vankeerberghen A, Boel A, Van Vaerenbergh K, De Beenhouwer H.** First case of *Staphylococcus pseudintermedius* infection in a human. J Clin Microbiol 2006, **44**, 4609-4612.
 29. **Weese JS, van Duijkeren E.** Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol 2010, **140**, 418-429.
 30. **Yoo JH, Yoon JW, Lee SY, Park HM.** High prevalence of Fluoroquinolone- and Methicillin-resistant *Staphylococcus pseudintermedius* isolates from canine pyoderma and otitis externa in veterinary teaching hospital. J Microbiol Biotechnol 2010, **20**, 798-802.
 31. **Yoon JW, Lee GJ, Lee SY, Park C, Yoo JH, Park HM.** Prevalence of genes for enterotoxins, toxic shock syndrome toxin 1 and exfoliative toxin among clinical isolates of *Staphylococcus pseudintermedius* from canine origin. Vet Dermatol 2010, **21**, 484-489.
 32. **Youn JH, Hwang SY, Kim SH, Koo HC, Shin S, Lim SK, Park YH.** *mecA* gene transferrability and antibiogram of zoonotic *Staphylococcus intermedius* from animals, staff and the environment in animal hospitals in Korea. J Microbiol Biotechnol 2010, **20**, 420-427.
 33. **Youn JH, Yoon JW, Koo HC, Lim SK, Park YH.** Prevalence and antimicrogram of *Staphylococcus intermedius* group isolates from veterinary staff, companion animals, and the environment in veterinary hospitals in Korea. J Vet Diagn Invest 2011, **23**, 268-274.