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Profiles of Enterotoxin Genes and Antimicrobial Resistance in *Staphylococcus pseudintermedius* Strains Isolated from Livestock and Companion Animals

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ABSTRACT - *Staphylococcus pseudintermedius* is an opportunistic pathogen in dogs and is recognized as a zoonotic pathogen causing public health concern. Although canine-associated *S. pseudintermedius* has mainly been recognized for its antimicrobial resistance and ability to cause skin infections in dogs, information on antimicrobial resistance profiles and enterotoxigenicity of *S. pseudintermedius* in livestock is very limited. In this study, we investigated the prevalence of 18 different staphylococcal enterotoxin (SE) genes and toxic shock syndrome toxin gene (*tst-1*) in *S. pseudintermedius* strains isolated from dogs, pigs, and beef cattle. Moreover, antimicrobial resistance profiles of the strains were determined along with the presence of *mecA* and SCC*mec* types. Except for one bovine isolate, all *S. pseudintermedius* isolates from dogs and pigs were resistant to multiple drugs (≥ 4 different drugs). Four out of six canine isolates were methicillin resistant and carried SCC*mec* type V. In addition, 11 different SE genes (*seb*, *sec*, *see*, *seg*, *sei*, *sej*, *sel*, *seo*, *sep*, *seq*, and *seu*) and *tst-1* were identified in *S. pseudintermedius* isolates from dogs, pigs, and beef cattle. Most *S. pseudintermedius* isolates (83%) harbored multiple SE genes, and *sel* (42%) and *sep* (42%) were most frequently detected in the isolates. Our results suggested that *S. pseudintermedius* isolates from livestock and companion animals may serve as a reservoir for SE genes and antimicrobial resistance.

Key words : *Staphylococcus pseudintermedius*, Antimicrobial resistance, Staphylococcal enterotoxins (SEs)

Staphylococcus pseudintermedius is an opportunistic pathogen, causing pyoderma, atoptic dermatitis, otitis externa, skin and soft tissue infections in domestic animals[1,2]. Although *S. pseudintermedius* has most frequently been isolated from the nares, mouth, and skin regions of healthy dogs and cats as well as from dogs and cats with skin infections[2-4], the carriage of *S. pseudintermedius* in other animals such as birds, horses, and goats has recently been reported[1,5,6]. Pilla *et al.* (2013) also reported the occurrence of *S. pseudintermedius* in bovine mastitis[7]. Since these animal-associated staphylococci can be transmitted by direct/indirect contacts with the animals or through consumption of foods of animal origin, *S. pseudintermedius* has raised food safety concerns over the past decades[8]. More recently, increased number of zoonotic infections has been reported in humans as antimicrobial resistance in *S. pseudintermedius* increases worldwide[9-12]. Methicillin-resistant *S. pseudintermedius* (MRSP) and multidrug-resistant (MDR) MRSP are of particular concern because β-lactam antibiotics are still the first choice of treatment for staphylococcal infections[13-15].

There are more than 20 different staphylococcal enterotoxins (SEs) that are functionally related and have similarities in sequences[16,17]. These SEs are known to be associated with food poisoning and toxic shock syndrome in humans[16-18]. Although the SEs have been identified in at least several species of staphylococci, *S. aureus* has most frequently been reported to be enterotoxigenic[16-20]. There are only few studies so far that described the presence of SE genes and toxic shock syndrome toxin gene (*tst-1*) in *S. pseudintermedius*, especially in antimicrobial resistant *S. pseudintermedius* strains from livestock and companion animals[7,21-23]. Thus, in this study, we determined and compared the occurrence of SE genes and *tst-1* in *S. pseudintermedius* strains isolated from dogs, pigs, and beef cattle. In addition, antimicrobial resistance profiles in the *S. pseudintermedius* strains were examined. Furthermore, the presence of *mecA* and staphylococcal cassette chromosome *mec* (SCC*mec*) types of *mecA*-positive *S. pseudintermedius* strains were determined along with their antimicrobial resistance profiles.

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Table 1. The characteristics of *S. pseudintermedius* strains isolated from dogs, pigs and beef cattle

Strain	Origin	Antimicrobial resistance profiles[1)]	*mecA*	SCC*mec*	OXA MICs (μg/mL)[2)]
SP-34[3)]	Dog	AMP-CHL-ENR-ERY-KAN-OXA-SXT-TET	+	V	2
SP-35		AMP-CHL-ERY-GEN-KAN-SXT-TET	-		0.25
SP-36[3)]		AMP-CHL-ERY-KAN-OXA-RIF-SXT-TET	+	V	>256
SP-37[3)]		AMP-CHL-ERY-GEN-KAN-OXA-SXT-TET	+	V	1.5
SP-38[3)]		AMP-CHL-ENR-KAN-OXA-SXT-TET	+	V	0.75
SP-39		AMP-CHL-ERY-GEN-KAN-SXT-TET	-		0.25
SP-242	Pig	ENR-ERY-KAN-SXT	-		0.125
SP-273		ENR-ERY-KAN-SXT	-		0.125
SP-111		ENR-ERY-KAN-SXT	-		0.25
SP-281		ENR-ERY-KAN-SXT	-		0.125
SP-362		CHL-ENR-ERY-KAN-SXT	-		0.125
SP-471	Cow	-			1

[1)] AMP, ampicillin; CHL, chloramphenicol; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; OXA, oxacillin; RIF, rifampin; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline
[2)] Oxacillin minimum inhibitory concentrations (MICs) indicate ≥ 0.5 μg/mL; methicillin-resistant *S. pseudintermedius* (MRSP) (OXA MICs ≥ 0.5 μg/mL) are by shading
[3)] indicates MRSP

Materials and methods

Bacterial isolation and identification

The 12 *S. pseudintermedius* isolates used in this study are listed in Table 1. A total of 401 swab samples were collected from dogs (n = 42), pigs (n = 190), and beef cattle (n = 169) between 2017 and 2018. Six canine-associated *S. pseudintermedius* strains were isolated from ear canals of dogs attending three different tertiary veterinary hospitals in Seoul, Seongnam, and Yongin. Five swine-associated *S. pseudintermedius* strains and one bovine-associated *S. pseudintermedius* strain were isolated from nasal swab samples from finishing pigs and beef cattle, respectively.

Each swab sample was inoculated onto Baired Parker Agar (BPA; Difco Laboratories) and incubated at 37ºC for 24-48 h. All putative staphylococcal colonies were selected and subcultured on BPA for identification. All *S. pseudintermedius* strains were identified using the Vitek 2 system (bioMérieux, Marcy-l'Étoile, France) and 16S rRNA sequencing method as described previously[24)]. In addition, the sequence of *tuf* gene was analyzed using a set of specific primers (Forward, 5'-GCCAGTTGAGGACGTATTCT-3'; Reverse, 5'-CCATTTCAGTACCTTCTGGTAA-3') to confirm *S. pseudintermedius* isolates[25)].

Antimicrobial susceptibility test

Susceptibilities to antimicrobial agents were determined using the disk diffusion methods according to the 2019 Clinical and Laboratory Standards Institute (CLSI) guidelines[26)]. The antimicrobial agents used were ampicillin (AMP, 10 μg), chloramphenicol (CHL, 30 μg), enrofloxacin (ENR, 5 μg), erythromycin (ERY, 15 μg), gentamicin (GEN, 30 μg), kanamycin (KAN, 30 μg), oxacillin (OXA, 1 μg), rifampin (RIF, 5 μg), sulfamethoxazole-trimethoprim (SXT, 23.73-1.25 μg) and tetracycline (30 μg) (BD Difco, Detroit, MI, USA). The minimum inhibitory concentrations (MICs) to oxacillin were determined by using micro-broth dilution method[26)].

Detection of antimicrobial resistant genes

Total genomic DNA samples were prepared from *S. pseudintermedius* as described previously[27)]. The presence of *mecA* gene was screened in all *S. pseudintermedius* strains, and SCC*mec* types were determined on *mecA*-positive *S. pseudintermedius* strains using the multiplex PCR method as described before[27)].

Detection of staphylococcal enterotoxin (SE) genes

A total of 19 different SE genes were detected in the 12 *S. pseudintermedius* strains as previously described[16,18)]. Briefly, the carriage of 5 classical SE genes (*sea*, *seb*, *sec*, *sed*, and *see*) and 13 newer SE genes (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu*) was examined by eight sets of multiplex PCR assays. Primers used for amplification of SE genes and their expected sizes are

Table 2. Primer sequences used for SEs, *tst-1*, and *mecA* genes PCR amplification

Genes	Primer	Oligonucleotide sequence (5'-3')	Size (bp)	PCR set	Reference
sea	F	CAGCATACTATATTGTTTAAAGGC	400	1-1	(18)
	R	CCTCTGAACCTTCCCATC			
seb	F	GTATGGTGGTGTAACTGAGCA	351	1-2	(18)
	R	TCAATCTTCACATCTTTAGAATCA			
sec	F	CTCAAGAACTAGACATAAAAGCTAGG	271	1-2	(39)
	R	TCAAAATCGGATTAACATTATCC			
sed	F	CTAGTTTGGTAATATCTCCTTTAAACG	319	1-1	(39)
	R	TTAATGCTATATCTTATAGGGTAAACATC			
see	F	CAGTACCTATAGATAAAGTTAAAACAAGC	178	1-2	(39)
	R	TAACTTACCGTGGACCCTTC			
seg	F	AAGTAGACATTTTTGGCGTTCC	287	2-1	(40)
	R	AGAACCATCAAACTCGTATAGC			
seh	F	GTCTATATGGAGGTACAACACT	213	2-2	(40)
	R	GACCTTTACTTATTTCGCTGTC			
sei	F	GGTGATATTGGTGTAGGTAAC	454	2-1	(40)
	R	ATCCATATTCTTTGCCTTTACCAG			
sej	F	ATAGCATCAGAACTGTTGTTCCG	152	2-1	(40)
	R	CTTTCTGAATTTTACCACCAAAGG			
sek	F	TAGGTGTCTCTAATAATGCCA	293	3-2	(40)
	R	TAGATATTCGTTAGTAGCTG			
sel	F	TAACGGCGATGTAGGTCCAGG	383	4-1	(40)
	R	CATCTATTTCTTGTGCGGTAAC			
sem	F	GGATAATTCGACAGTAACAG	379	3-2	(40)
	R	TCCTGCATTAAATCCAGAAC			
sen	F	CATCATGCTTATACGGAGGAG	301	4-2	(18)
	R	CCCACTGAACCTTTTACGTT			
seo	F	TGTGTAAGAAGTCAAGTGTAG	214	3-2	(40)
	R	TCTTTAGAAATCGCTGATGA			
sep	F	TGATTTATTAGTAGACCTTGG	381	2-2	(40)
	R	ATAACCAACCGAATCACCAG			
seq	F	TCAAGGAGTTAGTTCTGGAAATT	251	4-1	(18)
	R	GCTTACCATTGACCCAGAGA			
ser	F	GGATAAAGCGGTAATAGCAG	166	4-1	(40)
	R	GTATTCCAAACACATCTAAC			
seu	F	ATCAGAAACAAACATTAAAGCCCA	301	4-2	(18)
	R	TGACCATTTCCTTCGATAAACTTTAT			
tst-1	F	AAGCCCTTTGTTGCTTGCG	447	3-1	(39)
	R	ATCGAACTTTGGCCCATACTTT			
mecA	F	TGCTATCCACCCTCAAACAGG	286		(27)
	R	AACGTTGTAACCACCCCAAGA			

shown in Table 2. The multiplex PCR assays were carried out with eight different sets of mixtures using the following conditions: an initial denaturation at 95°C for 3 min; 30 cycles of denaturing at 95°C for 30s, annealing at 53°C for 45s, extension at 72°C for 40s; and a final enlongation at 72°C for 10 min. In addition, a singular PCR reaction for toxic shock syndrome toxin-1 (*tst-1*) gene was conducted as described before[18]. Mixture of genomic DNAs from reference *S. aureus* strains were used for positive controls for each PCR assay (COL: *seb*; FRI472: *sed*, *seg*, *sej*, *sel*, *sem*, *sen*, *seo*, *ser*, *seu*; FRI913: *sea*, *sec*, *see*, *sek*, *selq*, *tst1*; and MW2: *seh*; N135: *sei*, *sep*)[18].

Results and discussion

Although coagulase-positive *S. pseudintermedius* has

most frequently been isolated from both healthy dogs and dogs with skin infections[2-4], the colonization of *S. pseudintermedius* has also been observed in the other small animals and farm animals[1,5,6]. As shown in Table 1, prevalence of *S. pseudintermedius* in different animal species varied from 0.6 to 14.3%. Six, five, and one *S. pseudintermedius* strains were isolated from dogs (14.3%), pigs (3%), and beef cattle (0.6%), respectively. To the best of our knowledge, this is the first study to report swine-associated *S. pseudintermedius* strains in Korea, particularly MDR *S. pseudintermedius* strains.

As shown in Table 1, antimicrobial susceptibility assays revealed different antimicrobial resistance patterns among *S. pseudintermedius* isolates depending on origins of isolation. Interestingly, the six *S. pseudintermedius* isolates from dogs exhibited higher levels of MDR phenotype than other animal isolates. Of note, methicillin resistance was observed only in 4 canine-associated *S. pseudintermedius* isolates. These 4 MRSP isolates harbored SCC*mec* type V for methicillin resistance. The bovine isolate (SP-471) was susceptible to all the 11 antimicrobial agents tested, including OXA. In contrast to the SP-471, a previous study reported isolation of bovine-asssociated *S. pseudintermedius* from subclinical dairy cow mastitis with MDR phenotype, especially methicillin resistance[7]. Similar to the *S. pseudintermedius* isolates from dogs, five swine-associated *S. pseudintermedius* isolates exhibited MDR phenotype. Except for the single bovine isolate, all other *S. pseudintermedius* isolates from dogs and pigs displayed resistance to ENR, ERY, and SXT. These high levels of resistance to non-β-lactam antibiotic agents in *S. pseudintermedius* isolates from animals have also been described in previous studies[1,2,28]. In addition, in line with the previous studies, which reported >57% of CHL resistance[14,28-30], 7 out of 12 *S. pseudintermedius* isolates (58.3%) were resistant to CHL. Although methicillin resistance and highest level of MDR phenotype was observed only in canine-associated *S. pseudintermedius* isolates, continued monitoring of antimicrobial resistance in *S. pseudintermedius* isolates are necessary in major companion animals and farm animals.

Staphylococcal food poisoning cases acquired by eating enterotoxin-contaminated food are the one of the most commonly reported types of foodborne diseases worldwide[17]. The frequent incidence of food poisoning by staphylococci is in part due to the fact that staphylococci, such as *S. aureus*, can grow over a wide range of hosts and environments[31]. Although the majority of studies related to SEs have been associated with *S. aureus*, several groups have reported that *S. pseudintermedius* strains isolated from dogs produce SEs. Aside from dogs, very few information is available for *S. pseudintermedius* isolates from other animals, especially food-producing animals. As shown in Table 3, 11 different SE genes (*seb*, *sec*, *see*, *seg*, *sei*, *sej*, *sel*, *seo*, *sep*, *seq*, and *seu*) and *tst-1* were identified in *S. pseudintermedius* strains isolated from dogs, pigs, and beef cattle. Except for one isolate (SP-281), all *S. pseudintermedius* isolates harbored at least one of the 19 SE genes. Although the bovine isolate (SP-471) were susceptible to all antimicrobial agents tested, SP-471 carried highest number of SE genes (9 SE genes) among the 12 *S. pseudintermedius* isolates. It has been reported that the most common SEs are SEA and SEB in staphylococci-related food poisoning[16,17,19]. Previous studies also reported that canine-associated *S. pseudintermedius* isolates most frequently carry *sec*[21-23,32]. However, none of the 12 *S. pseudintermedius* isolates carried *sea* and only 2/12 (16.7%) *S. pseudintermedius* isolates were positive for *seb*. In addition, only 2/6 canine-associated *S. pseudintermedius* isolates were positive for *sec*. Although these *sec* genes were not sequenced for further analysis, it has been reported that canine-associated *S. pseudintermedius* often produce SEC variant (SEC_{canine})[33]. In addition to the SEC_{canine}, several antigenic variants of SEC (i.e. SEC_{bovine}, SEC_{ovine}, SEC1-3) have also been reported[21,32]. Interestingly, *see* and *seq* genes were detected only in canine isolates, indicating that these genes might be host specific. Structure of SEE is similar to SEA and has been reported in some cases of food poisoning[17,34]. Tanabe *et al*. (2013) also reported presence of *seq* in *S. pseudintermedius* strains isolated from dogs[23]. Among the 12 SE genes detected, *sel* and *sep* were each identified in 5 *S. pseudintermedius* isolates, resulting in highest prevalence rate (41.7%) among the 19 different SE genes. Previously, SEL was detected in a pathogenicity island of bovine mastitis *S. aureus* (SaPIbov) isolate exhibiting a variety of biological activities including superantigenic, pyrogenic, and endotoxigenic activity in a rabbit model[35]. SEP was also characterized in a MRSA strain isolated from a human bacteremia case[36]. In addition to SEs, toxic shock syndrome toxin (TSST-1), encoded by *tst-1*, has been well studied as a non-specific T-cells activator and inducer of fatal toxic shock syndrome, although TSST-1 lacks emetic activity[16,17,37]. While all 6 *S. pseudintermedius* isolates from dogs were negative for *tst-1*, four isolates from pigs and one isolate from beef cattle were found to be positive for *tst-1* gene. In a previous study, Hu *et al.* (2008) showed that *sec*, *seg*, *sei*, *sel*, *sem*, *seo*, and *tst-1* genes were frequently associated with SCC*mec* type I and type II[38]. As shown in Table 3, only the four MRSP isolates which carries SCC*mec* type V had *see* or *seq* genes. These results indicate that prevalence of SE genes and *tst-1* among *S. pseudintermedius* isolates may be associated with host factors in different animals and environmental factors.

Table 3. The prevalence of SEs and *tst-1* genes from *S. pseudintermedius*

Strain (No. of isolates)	Origin	Number of SE genes																		
		sea	*seb*	*sec*	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*	*sel*	*sem*	*sen*	*seo*	*sep*	*seq*	*ser*	*seu*	*tst-1*
SP-34	Dog					1										1				
SP-35						1											1			
SP-36				1								1								
SP-37						1										1	1			
SP-38																	1			
SP-39				1					1			1								
SP-242	Pig			1						1										1
SP-273							1													1
SP-111			1	1			1			1		1				1			1	1
SP-281																				
SP-362									1	1		1			1	1				
SP-471	Cow		1				1		1	1		1			1	1			1	1
Total			2	4		3	3		3	4		5			2	5	3		2	4

In conclusion, our results indicate that *S. pseudintermedius* strains isolated from livestock and companion animals became resistant to multiple antimicrobial agents used to treat infections in humans. Although methicillin resistance was observed only in canine isolates, all the pig isolates were resistant to at least 4 different antimicrobial agents. In addition to antimicrobial resistance, most *S. pseudintermedius* isolates carried multiple SE genes and *tst-1* that can cause food-producing in humans. Our results also indicated that *S. pseudintermedius* isolates from livestock and companion animals may serve as a reservoir for staphylococcal enterotoxin genes.

국문요약

*Staphylococcus pseudintermedius*는 개에서 기회감염을 유발하는 병원체이며, 공중보건학적으로도 주요한 인수공통 병원체이다. 개에서 분리된 *S. pseudintermedius* 균주들은 주로 항생제 내성 및 개에서 피부 감염을 유발하는 주요 원인균으로 연구되어 왔지만, 가축에서 분리된 *S. pseudintermedius* 균주들의 항생제 내성 및 장내 독소 생성에 대한 정보는 매우 제한적이다. 본 연구에서는 개, 돼지, 육우에서 분리된 *S. pseudintermedius* 균주들에서 18가지의 장내 독소 (staphylococcal enterotoxin; SE) 유전자와 toxic shock syndrome toxin 유전자(*tst-1*)의 분포양상을 조사하였다. 또한, *S. pseudintermedius* 균주들의 항생제 내성 양상과 더불어 *mecA* 유전자 및 SCC*mec* type 또한 확인하였다. 육우에서 분리한 하나의 균주를 제외한 모든 개와 돼지 분리주 들이 4개 이상의 항생제에 내성을 보였으며, 개에서 분리된 6개의 균주 중 4개의 *S. pseudintermedius* 균주들이 메티실린 내성과 더불어 SCC*mec* V를 가진 것으로 확인 되었다. 총 11개의 SE 유전자들 (*seb, sec, see, seg, sei, sej, sel, seo, sep, seq, seu*) 및 *tst-1*가 개, 돼지 및 육우로부터 분리된 *S. pseudintermedius* 균주들에서 확인 되었으며, 대부분의 분리주들 (83%)에서 2개 이상의 SE 유전자들이 확인 되었고, 그 중 *sel* (42%) 및 *sep* (42%)가 가장 빈번하게 검출 되었다.

본 연구를 통하여 반려견에서 뿐만 아니라 주요 가축에서 존재하는 *S. pseudintermedius* 균주들에서 높은 항생제 내성 양상을 확인 하였으며 , 항생제 내성과 더불어 여러 staphylococcal enterotoxin 및 *tst-1* 유전자들을 전파할 가능성을 확인 하였다 .

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References

1. Ruscher, C., Lubke-Becker, A., Wleklinski, C.G., Soba, A., Wieler, L.H., Walther, B., Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. *Vet Microbiol.* **136**(1-2), 197-201 (2009).
2. Weese, J.S., van Duijkeren, E., Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in

veterinary medicine. *Vet Microbiol*. **140**(3-4), 418-29 (2010).
3. Penna, B., Mendes, W., Rabello, R., Lilenbaum, W., Carriage of methicillin susceptible and resistant *Staphylococcus schleiferi* among dog with or without topic infections. *Vet Microbiol*. **162**(1), 298-9 (2013).
4. Penna, B., Varges, R., Medeiros, L., Martins, G.M., Martins, R.R., Lilenbaum, W., Species distribution and antimicrobial susceptibility of staphylococci isolated from canine otitis externa. *Vet Dermatol*. **21**(3), 292-6 (2010).
5. Biberstein, E.L., Jang, S.S., Hirsh, D.C., Species distribution of coagulase-positive staphylococci in animals. *J. Clin Microbiol*. **19**(5), 610-5 (1984).
6. Futagawa-Saito, K., Suzuki, M., Ohsawa, M., Ohshima, S., Sakurai, N., Ba-Thein, W., Fukuyasu, T., Identification and prevalence of an enterotoxin-related gene, *se-int*, in *Staphylococcus intermedius* isolates from dogs and pigeons. *J. Appl Microbiol*. **96**(6), 1361-6 (2004).
7. Pilla, R., Bonura, C., Malvisi, M., Snel, G.G., Piccinini, R., Methicillin-resistant *Staphylococcus pseudintermedius* as causative agent of dairy cow mastitis. *Vet Rec*. **173**(1), 19 (2013).
8. Kadariya, J., Smith, T.C., Thapaliya, D., *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *Biomed Res Int*. **2014**, 827965 (2014).
9. van Duijkeren, E., Kamphuis, M., van der Mije, I.C., Laarhoven, L.M., Duim, B., Wagenaar, J.A., Houwers, D.J., Transmission of methicillin-resistant *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics. *Vet Microbiol*. **150**(3-4), 338-43 (2011).
10. Soedarmanto, I., Kanbar, T., Ulbegi-Mohyla, H., Hijazin, M., Alber, J., Lammler, C., Akineden, O., Weiss, R., Moritz, A., Zschock, M., Genetic relatedness of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolated from a dog and the dog owner. *Res Vet Sci*. **91**(3), e25-7 (2011).
11. Somayaji, R., Priyantha, M.A., Rubin, J.E., Church, D., Human infections due to *Staphylococcus pseudintermedius*, an emerging zoonosis of canine origin: report of 24 cases. *Diagn Microbiol Infect Dis*. **85**(4), 471-6 (2016).
12. 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*. **44**(12), 4609-12 (2006).
13. Gronthal, T., Eklund, M., Thomson, K., Piiparinen, H., Sironen, T., Rantala, M., Antimicrobial resistance in *Staphylococcus pseudintermedius* and the molecular epidemiology of methicillin-resistant *S. pseudintermedius* in small animals in Finland. *J. Antimicrob Chemother.* **72**(4), 1021-30 (2017).
14. Bond, R., Loeffler, A., What's happened to *Staphylococcus intermedius*? Taxonomic revision and emergence of multidrug resistance. *J. Small Anim Pract*. **53**(3), 147-54 (2012).
15. Thakuria, B., Lahon, K., The beta lactam antibiotics as an empirical therapy in a developing country: an update on their current status and recommendations to counter the resistance against them. *J. Clin Diagn Res*. **7**(6), 1207-14 (2013).
16. Fisher, E.L., Otto, M., Cheung, G.Y.C., Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. *Front Microbiol*. **9**, 436 (2018).
17. Balaban, N., Rasooly, A., Staphylococcal enterotoxins. *Int J Food Microbiol*. **61**(1), 1-10 (2000).
18. Park, J.Y., Fox, L.K., Seo, K.S., McGuire, M.A., Park, Y.H., Rurangirwa, F.R., Sischo, W.M., Bohach, G.A., Detection of classical and newly described staphylococcal superantigen genes in coagulase-negative staphylococci isolated from bovine intramammary infections. *Vet Microbiol*. **147**(1-2), 149-54 (2011).
19. Podkowik, M., Park, J.Y., Seo, K.S., Bystron, J., Bania, J., Enterotoxigenic potential of coagulase-negative staphylococci. *Int J Food Microbiol*. **163**(1), 34-40 (2013).
20. Zhang, Y., Wang, Y., Cai, R., Shi, L., Li, C., Yan, H., Prevalence of enterotoxin genes in *Staphylococcus aureus* isolates from pork production. *Foodborne Pathog Dis*. **15**(7), 437-43 (2018).
21. Yoon, J.W., Lee, G.J., Lee, S.Y., Park, C., Yoo, J.H., Park, H.M., Prevalence of genes for enterotoxins, toxic shock syndrome toxin 1 and exfoliative toxin among clinical isolates of *Staphylococcus pseudintermedius* from canine origin. *Vet Dermatol*. **21**(5), 484-9 (2010).
22. Phumthanakorn, N., Fungwithaya, P., Chanchaithong, P., Prapasarakul, N., Enterotoxin gene profile of methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs, humans and the environment. *J. Med Microbiol*. **67**(6), 866-73 (2018).
23. Tanabe, T., Toyoguchi, M., Hirano, F., Chiba, M., Onuma, K., Sato, H., Prevalence of staphylococcal enterotoxins in *Staphylococcus pseudintermedius* isolates from dogs with pyoderma and healthy dogs. *Microbiol Immunol*. **57**(9), 651-4 (2013).
24. Forsman, P., Tilsala-Timisjarvi, A., Alatossava, T., Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S-23S rRNA spacer regions. *Microbiology.* **143**(11), 3491-500 (1997).
25. Khosravi, A.D., Roointan, M., Abbasi Montazeri, E., Aslani, S., Hashemzadeh, M., Taheri Soodejani, M., Application of *tuf* gene sequence analysis for the identification of species of coagulase-negative staphylococci in clinical samples and evaluation of their antimicrobial resistance pattern. *Infect Drug Resist*. **11**, 1275-82 (2018).
26. Clinical and Laboratory Standards Institute. 2019 Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, VET08, **38**.
27. Kondo, Y., Ito, T., Ma, X.X., Watanabe, S., Kreiswirth, B.N., Etienne, J., Hiramatsu, K., 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.* **51**(1), 264-74 (2007).
28. Cain, C.L., Antimicrobial resistance in staphylococci in small animals. *Vet Clin North Am Small Anim Pract*. **43**(1),

19-40 (2013).
29. Perreten, V., Kadlec, K., Schwarz, S., Gronlund Andersson, U., Finn, M., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bemis, D.A., Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J.A., van Duijkeren, E., Weese, J.S., Fitzgerald, J.R., Rossano, A., Guardabassi, L., Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. *J. Antimicrob Chemother*. **65**(6), 1145-54 (2010).
30. Kadlec, K., Schwarz, S., Perreten, V., Andersson, U.G., Finn, M., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bemis, D.A., Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J.A., van Duijkeren, E., Weese, J.S., Fitzgerald, J.R., Rossano, A., Guardabassi, L., Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. *J. Antimicrob Chemother*. **65**(8), 1826-8 (2010).
31. Chon, J.W., Sung, K., Khan, S., 2017 Methicillin-resistant *Staphylococcus aureus* (MRSA) in food- producing and companion animals and food products. **Chapter 3**.
32. Edwards, V.M., Deringer, J.R., Callantine, S.D., Deobald, C.F., Berger, P.H., Kapur, V., Stauffacher, C.V., Bohach, G.A., Characterization of the canine type C enterotoxin produced by *Staphylococcus intermedius* pyoderma isolates. *Infect Immun*. **65**(6), 2346-52 (1997).
33. Cardona, I.D., Cho, S.H., Leung, D.Y., Role of bacterial superantigens in atopic dermatitis : implications for future therapeutic strategies. *Am J Clin Dermatol*. **7**(5), 273-9 (2006).
34. Van den Bussche, R.A., Lyon, J.D., Bohach, G.A., Molecular evolution of the staphylococcal and streptococcal pyrogenic toxin gene family. *Mol Phylogenet Evol*. **2**(4), 281-92 (1993).
35. Orwin, P.M., Fitzgerald, J.R., Leung, D.Y., Gutierrez, J.A., Bohach, G.A., Schlievert, P.M., Characterization of *Staphylococcus aureus* enterotoxin L. *Infect Immun*. **71**(5), 2916-9 (2003).
36. Calderwood, M.S., Desjardins, C.A., Sakoulas, G., Nicol, R., Dubois, A., Delaney, M.L., Kleinman, K., Cosimi, L.A., Feldgarden, M., Onderdonk, A.B., Birren, B.W., Platt, R., Huang, S.S., Program CDCPE. Staphylococcal enterotoxin P predicts bacteremia in hospitalized patients colonized with methicillin-resistant *Staphylococcus aureus*. *J. Infect Dis*. **209**(4), 571-7 (2014).
37. Durand, G., Bes, M., Meugnier, H., Enright, M.C., Forey, F., Liassine, N., Wenger, A., Kikuchi, K., Lina, G., Vandenesch, F., Etienne, J., Detection of new methicillin-resistant *Staphylococcus aureus* clones containing the toxic shock syndrome toxin 1 gene responsible for hospital- and community-acquired infections in France. *J. Clin Microbiol*. **44(3)**, 847-53 (2006).
38. Hu, D.L., Omoe, K., Inoue, F., Kasai, T., Yasujima, M., Shinagawa, K., Nakane, A., Comparative prevalence of superantigenic toxin genes in meticillin-resistant and meticillin-susceptible *Staphylococcus aureus* isolates. *J. Med Microbiol*. **57**(9), 1106-12 (2008).
39. Becker, K., Roth, R., Peters, G., Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol*. **36**(9), 2548-53 (1998).
40. Omoe, K., Hu, D.L., 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*. **246**(2), 191-8 (2005).