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Prevalence and risk factors of *Staphylococcus* spp. carriage among dogs and their owners: A cross-sectional study

Jae-Ik Han [a,b,1], Cheol-Ho Yang [b,1], Hee-Myung Park [b,*]

[a] Laboratory of Wildlife Diseases, College of Veterinary Medicine, Chonbuk National University, Iksan 54596, South Korea
[b] Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 143-701, South Korea

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ABSTRACT

This study investigated colonization and association of staphylococci between healthy dogs and their owners. In a cross-sectional study, nasal carriage and antibiotic susceptibility of isolated staphylococci were determined for 119 dogs and 107 owners. Relatedness of the *Staphylococcus* isolates in dogs and their owners was investigated using antibiograms, toxin profiles, and genotyping by pulsed-field gel electrophoresis (PFGE), multilocus sequence type, and *spa* typing. Risk factors for carriage of methicillin-resistant staphylococci in dogs were also evaluated. *Staphylococcus* spp. were isolated from 65 (60.7%) owners and 44 (37.0%) dogs. The following species were isolated, listed in order of decreasing frequency: *S. epidermidis*, *S. pseudintermedius*, *S. aureus*, *S. scheiferi* subsp. *coagulans*, *S. haemolyticus*, *S. sciuri*, *S. saprophyticus* and *S. warneri*. *S. pseudintermedius* (65.9%) was the major isolate in dogs while *S. epidermidis* (81.5%) was the major type in owners. Among the isolates, 71.6% were methicillin resistant (MR) and 95.4% of the isolates demonstrated multi-drug resistance regardless of the origin. Only one dog–owner pair shared the same *Staphylococcus* spp. (*S. pseudintermedius*); however, the organisms were of different PFGE subtypes and exhibited different antibiotic resistance and toxin profiles while both isolates displayed same sequence type (ST365). While the dog-origin isolate showed *spa* type t02, the owner-origin isolate was negative to PCRs targeting *spa* gene sequence. Risk factor analysis showed that the presence of cohabitant animals was correlated with the nasal carriage of MR staphylococci in dogs. The cumulative data indicated that animal- and owner-origin staphylococci have various subtypes with high prevalence of MR; however, the bacteria are not shared between healthy dogs and their owners.

Introduction

Staphylococci are part of the normal microflora of the skin and mucosal surfaces of the upper respiratory tract of humans and animals. Coagulase-positive staphylococci (CPS) are important pathogens that cause numerous diseases in animals (Lyskova et al., 2007). Among them, *Staphylococcus pseudintermedius* has been shown to be both a normal inhabitant and an opportunistic pathogen of the skin, ear and other tissues in dogs (Devriese et al., 2009). In addition, it has been reported that *S. pseudintermedius* is a potential invasive pathogen found in dog-bite wounds in humans (Lee, 1994). In some geographical areas, *S. schleiferi* is emerging as a common pathogenic species of canine pyoderma and otitis (Cain et al., 2011).

As the clinical use of antibiotics has increased, staphylococci, particularly the strains associated with nosocomial infection, have shown rapid acquisition of resistance to commonly used antibiotics (Yoon et al., 2010a). In the development and spread of the resistance, plasmids likely play an important role by acting as carriers of resistance genes or as vectors for transposon-borne resistance genes (Malik et al., 2005). Several studies have indicated that staphylococci with the same staphylococcal cassette chromosome *mec* (SCC*mec*) subtype or genetically identical *Staphylococcus* spp. can be found among veterinary hospital staff and animals (Boost et al., 2008; Morris et al., 2010; Youn et al., 2011; Moon et al., 2012); however, in South Korea it has not yet been verified whether owners and their companion dogs share genetically identical *Staphylococcus* spp.

To determine if the same *Staphylococcus* spp. occur in owners and their healthy dogs, we first evaluated the prevalence of nasal staphylococci and performed genotyping of the isolates from dog–owner pairs. In addition, we examined antibiotic resistance and toxin profiles of the isolated staphylococci and analyzed the risk factors for nasal carriage by methicillin-resistant (MR) staphylococci in dogs.

Materials and methods

Sample collection

The procedures and sample handing in this study were approved by the Institutional Animal Care and Use Committee (IACUC; approval number KU13087; date of approval 01 Jun 2013). From May 2013 to Feb 2014, 107 adult owners and their

* Corresponding author. Tel.: +82 2450 4140.
E-mail address: parkhee@konkuk.ac.kr (H.-M. Park).
[1] These authors contributed equally to this work.

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Table 1
Primers used in the study.

Purpose	Target	Primer	Sequence (5′ to 3′)	Annealing temperature (°C)	Reference
Identification	16S ribosomal RNA	27F	AGAGTTTGATCCTGGCTCAG	60	Lane et al. (1985)
		1492R	GGTTACCTTGTTACGACTT		
	Heat shock protein 60	H279	GAIIIIGCIGGIGAYGGIACIACIAC	56	Hill et al. (2006)
		H280	YKIYKITCICCRAAICCIGGIGCYTT		
		H1612	GAIIIIGCIGGYGACGGYACSACSAC		
		H1613	CGRCGRTCRCCGAAGCCSGGIGCCTT		
	S. epidermidis	Fwd	ATCAAAAAGTTGGCGAACCTTTTCA	55	Martineau et al. (2000)
		Rev	CAAAAGAGCGTGGAGAAAAGTATCA		
	S. aureus	au-F3	TCGCTTGCTATGATTGTGG	56	Sasaki et al. (2010)
		au-nucR	GCCAATGTTCTACCATAGC		
	S. pseudintermedius	pse-F2	TRGGCAGTAGGATTCGTTAA	56	
		pse-R5	CTTTTGTGCTYCMTTTTGG		
	S. schleiferi	sch-F	AATGGCTACAATGATAATCACTAA	56	
		sch-R	CATATCTGTCTTTCGGCGCG		
spa typing	*spa*	SIspaF	AACCTGCGCCAAGTTTCGATGAAG	60	Moodley et al. (2009)
		SIspaR	CGTGGTTTGCTTTAGCTTCTTGGC		
		SPspa1F	CCGCTCTATTTTTAGGTTAATC	60	Perreten et al. (2010)
		SIspaFlkR1	CGTAACAACTCAATGCTACATA		
MLST	*tuf*	Forward	CAATGCCACAAACTCG	60	Solyman et al. (2013)
		Reverse	GCTTCAGCGTAGTCTA		
	cpn60	Forward	GCGACTGTACTTGCACAAGCA		
		Reverse	AACTGCAACCGCTGTAAATG		
	pta	Forward	GTGCGTATCGTATTACCAGAAGG		
		Reverse	GCAGAACCTTTTGTTGAGAAGC		
	purA	Forward	GATTACTTCCAAGGTATGTTT		
		Reverse	TCGATAGAGTTAATAGATAAGTC		
	fdh	Forward	TGCGATAACAGGATGTGCTT		
		Reverse	CTTCTCATGATTCACCGGC		
	ack	Forward	CACCACTTCACAACCCAGCAAACT		
		Reverse	AACCTTCTAATACACGCGCACGCA		
	sar	Forward	GGATTTAGTCCAGTTCAAAATTT		
		Reverse	GAACCATTCGCCCCATGAA		
*mec*A PCR	*mec*A	MecA147-F	CCCTTTTTATACAATCTCGTT		Zhang et al. (2005)
		MecA147-R	ATATCATCTGCAGAATGGG		
Toxin profiles	*sea*	SEA-3	CCTTTGGAAACGGTTAAAACG	55	Yoon et al. (2010a)
		SEA-4	TCTGAACCTTCCCATCAAAAAC		
	seb	SEB-1	TCGCATCAAACTGACAAACG	55	
		SEB-4	GCAGGTACTCTATAAGTGCCTGC		
	sec	SEC-3	CTCAAGAACTAGACATAAAAGCTAGG	55	
		SEC-4	TCAAAATCGGATTAACATTATCC		
	sed	SED-3	CTAGTTTGGTAATATCTCCTTTAAACG	55	
		SED-4	TTAATGCTATATCTTATAGGGTAAACATC		
	see	SEE-3	CAGTACCTATAGATAAAGTTAAAACAAGC	55	
		SEE-2	TAACTTACCGTGGACCCTTC		
	tsst-1	TST-3	AAGCCCTTTGTTGCTTGCG	55	
		TST-6	ATCGAACTTTGGCCCATACTTT		
	siet	siet1	ATGGAAAATTTAGCGGCATCTGG	56	
		siet2	CCATTACTTTTCGCTTGTTGTGC		

119 dogs were recruited at five veterinary hospitals in the Seoul and Gyeounggi provinces, South Korea. Nine owners brought two dogs each and one owner brought four dogs. As the study focused on carriage rather than infection, we excluded seriously ill dogs, and those with obvious infections.

The nasal swab samples were collected using the Transystem Culture swab transport system (Copan). The sterile wet swab was inserted into the nares and gently rotated to make contact with the nasal septum. After collection, each sample was immediately transferred to the laboratory and cultured for isolation of staphylococci. The following animal information was obtained from the owners: sex, age, and species of their dogs; medication history; housing; frequency and location of walks; and the presence of cohabitant animals (i.e., dogs, cats, and exotic animals). Information about the owners, including the age, sex, and medication history of family members, and their average time spent in contact with the dog, was also collected.

Isolation and identification of staphylococci

The swab samples were incubated onto trypticase soy agar plates containing 5% sheep blood at 37 °C for 24–48 h. After incubation, a colony resembling staphylococci was identified based on the colony morphology, complete or incomplete hemolysis, Gram-staining, and a conventional catalase test. The *Staphylococcus* isolates were further tested for coagulase synthesis and DNase production.

The species of CPS and coagulase-negative (CNS) staphylococci were identified by molecular analysis. After bacterial DNA extraction, the 16S ribosomal RNA gene and heat shock protein 60 were amplified as described previously (Table 1) (Lane et al., 1985; Hill et al., 2006). After sequencing of the amplicon, the sequence homology of deduced nucleotide sequences to the *Staphylococcus* spp. was analyzed with the BLAST search program (National Center for Biotechnology Information)[1] based on the Clinical and Laboratory Standards Institute (CLSI) guideline (MM-18A). Finally, the species identification for the isolates was confirmed using the multiplex PCR method (Sasaki et al., 2010). The *S. aureus* ATCC 25923 and a clinical isolate of *S. epidermidis*, confirmed by species-specific PCR (Martineau et al., 2000) and sequencing, were used as the positive and negative controls.

PFGE and MLST

In cases of dog–owner pairs that displayed concurrent detection of *S. pseudintermedius*, PFGE, MLST, and *spa* typing were performed to identify whether they shared the genetically identical strain as described previously (Murchan et al., 2003; Moodley et al., 2009; Perreten et al., 2010; Solyman et al., 2013). In MLST and *spa* typing, the amplicons were sequenced to compare its sequence to the corresponding database to determine the sequence type of the isolate.

Determination of methicillin resistance

The MR of isolated staphylococci was identified in three ways: (1) a Kirby–Bauer disc diffusion test using a 1 μg oxacillin disc or 30 μg cefoxitin disc (Oxoid)

[1] See: http://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed 25 September 2014).