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Distribution of Major Staphylococcal Cassette Chromosome *mec* Types and Exfoliative Toxin Genes in *Staphylococcus pseudintermedius* Strains from Dogs with Superficial Pyoderma in Japan

Tomoko KASAI[1,3], Yukio KATO[2], Sanae SAEGUSA[3], Masaru MURAKAMI[1]

[1]Laboratory of Molecular Biology, [2]Laboratory of Public Health 2, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuou-ku, Sagamihara, Kanagawa 252-5201 Japan
[3]Kitagawa Veterinary Hospital, 1-39-1 Minamitokiwadai, Itabashi-ku, Tokyo, 174-0072 Japan

Abstract: *Staphylococcus pseudintermedius* is a major pathogen of canine pyoderma, known to produce exfoliative toxins that could be involved in formation of cutaneous lesions. To understand the genotypic distribution of *S. pseudintermedius*, we surveyed 74 dogs with pyoderma in three veterinary hospitals in Japan. Seventy-four *S. pseudintermedius* strains were isolated, 52 of which (70.3%) were *mecA*-positive methicillin-resistant *S. pseudintermedius* (MRSP). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of the identified MRSP strains revealed that the most prevalent genotype was type III-like (63.4%) followed by type V (34.6%). These data suggest high prevalence of MRSP strains consisting of two major SCC*mec* types among canine pyoderma in Japan. We found low prevalence of exfoliative toxin genes (*exp*) in the MRSP strains: *expA* and *expB* were present in 1.9% and 0%, respectively. These findings suggest no association in carriage between *mecA* and *exp* genes in *S. pseudintermedius* from canine pyoderma.

Key words: *Staphylococcus pseudintermedius*; superficial pyoderma; methicillin resistance; SCC*mec*; exfoliative toxin

Introduction

Staphylococcus pseudintermedius is a normal inhabitant of the skin and mucosae of dogs[1, 2]. This species is also known to be the major pathogen of superficial pyoderma, one of the most common infectious diseases of canine cutaneous disorder[3]. Previous studies have revealed that *S. pseudintermedius* possess virulence factors such as exfoliative toxins (ETs) ExpA and B, which cause skin exfoliation[4-6]. However, few studies have described the distribution of ETs in *S. pseudintermedius* from canine superficial pyoderma[4, 5], and the presence of ETs in methicillin-resistant *S. pseudintermedius* (MRSP) has not been reported.

Since the first report of a *mecA*-positive MRSP strain in 1999[7], MRSP infections have been increasing in small animal medicine[8-12]. According to previous studies, MRSP strains are mainly classified into two genotypes based on the type of staphylococcal cassette chromosome *mec* (SCC*mec*): SCC*mec* type III-like clones (informally designated type II-III by Descloux *et al.*[13]), which are found in Europe and many other areas across the world[14-18], and type V clones, which are prevalent in North America, Korea and Thailand[14, 15, 19-22]. Genotyping is important and helpful in understanding the geographic distribution and estimating the epidemic nature and spread of MRSP clones. However, only a few studies have performed genotype-based analysis of canine

superficial pyoderma caused by MRSP in Japan[18]. We therefore conducted molecular analysis of MRSP strains isolated from canine superficial pyoderma and determined SCC*mec* types in Japan. We also analyzed two exfoliative toxin genes, *expA* and *expB*, to investigate the association between methicillin resistance and the carriage of toxin genes. Here, we describe the prevalence of methicillin resistance and exfoliative toxin genes in the genome of *S. pseudintermedius* among 74 dogs with superficial pyoderma from three veterinary hospitals in Japan.

Materials and Methods

Sample collecting

We examined 74 dogs with superficial pyoderma in three private veterinary hospitals in three prefectures of Japan between April 2010 and December 2012. The 74 dogs (37 males, 37 females; mean age, 7.9 years [range, 10 months to 15 years]) were 10 Shih Tzu dogs, 9 French Bulldogs, 9 Poodles, 7 Miniature Dachshunds, 5 Shiba Inu dogs, 5 Pugs, 4 Chihuahuas, 4 Cocker Spaniels, 2 West Highland white terriers, 2 Retrievers, 2 Malteses, 2 Yorkshire terriers, 2 Cavalier King Charles Spaniels, 2 Jack Russells, 1 Basset, 1 Chin, 1 German shepherd, 1 Pekingese, 1 Schnauzer, 1 Weimaraner and 3 Mixed breeds. A total of 74 specimens were collected by swabbing skin lesions. Bacterial strains from the specimens were cultivated on tryptic soy agar containing 5% sheep blood (BD Japan, Co., Ltd., Tokyo, Japan) at 37°C for 18 h. All strains were identified as staphylococci based on colony morphology, Gram stain appearance and the catalase test.

Species identification, determination of methicillin resistance and SCC*mec* typing

Crude DNA extraction from a single colony and staphylococcal species identification using multiplex PCR (M-PCR) were performed as previously described by Sasaki *et al.*[23].

To identify methicillin resistance, a PCR method[24] for detection of the *mecA* gene was used. Subsequently, SCC*mec* typing of the MRSP strains identified was performed. To discriminate SCC*mec* types I to V, including type III-like, classified based on the *ccr* and *mec* gene complexes, two M-PCRs[25] and one duplex PCR[15] were carried out.

Detection of exfoliative toxin genes

Fragments from two exfoliative toxin genes, *expA* and *expB*, were amplified by conventional PCR. The oligonucleotide primers were as previously reported (Yamamoto *et al.*, 2012, 15th Annual meeting of The Japanese Society of Veterinary Dermatology): 5´-ATTTGTTCACATGGATTTATT-3´ (forward) and 5´-AGGGGCATTAACAATAAGATC-3´ (reverse) for *expA*, and 5´-TTTATGACAGCTATGCTCATT-3´ (forward) and 5´-TCCTAAATTAGCGTCAAAAAT-3´ (reverse) for *expB*. The thermal cycling parameters consisted of an initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, with an additional final extension step of 72°C for 2 min. PCR products were separated on 1.0% agarose gel with TAE buffer and visualized with ethidium bromide.

Results and Discussion

A total of 74 staphylococci from 74 dogs with superficial pyoderma were obtained. All strains were identified as *S. pseudintermedius* as previously described[23]. Molecular characteristics of the isolated *S. pseudintermedius* were investigated using several conventional PCR methods as previously described[15, 25]. As shown in Table 1, MRSP with *mecA* accounted for 70.3% (52/74) of the strains. This frequency was very high compared to previous surveillance data from other countries: 5.1% in the UK[26], 29.3% in Korea[20] and 47.8% in North China[17], and was similar to the previously reported 66.5% in Japan[27]. This indicates a high prevalence of MRSP in canine superficial pyoderma in Japan. Sixty-five (87.8%) out of 74 dogs had been treated with antimicrobials previously: 50 (96.2%) of 52 dogs infected with MRSP and 15 (68.2%) of 22 dogs infected with MSSP. Dogs infected with MRSP tended to have greater exposure to antibiotics than MSSP-infected dogs.

Table 1 Distribution of methicillin-resistant *S. pseudintermedius* strains from canine pyoderma

	No. of strains (%)
S. pseudintermedius	74 (100)
Methicillin-susceptible	22 (29.7)
Methicillin-resistant	52 (70.3)
SCC*mec*-type III-like	33
SCC*mec*-type V	18
Nontypeable	1

Table 2 Distribution of two exfoliative toxin genes among *S. pseudintermedius* strains

S. pseudintermedius	n	No. of positive strains (%)	
		expA	*expB*
Methicillin-susceptible	22	5 (22.7)	1 (4.5)
Methicillin-resistant	52	1 (1.9)	0 (0)

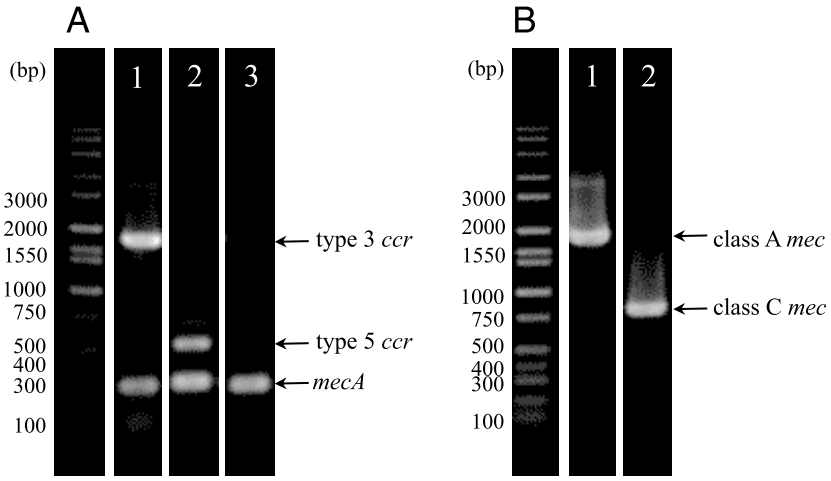

Fig. 1 Multiplex PCR analysis of *ccr* gene complex (A) and *mec* gene complex (B).
Lane 1, SCC*mec* type III-like MRSP; lane 2, SCC*mec* type V MRSP; lane 3, SCC*mec* nontypeable MRSP. (A) Upper bands in lanes 1 and 2 represent types 3 and 5 *ccr* genes, respectively. Lane 3 is a nontypeable strain that possesses *mecA* gene only. (B) Lanes 1 and 2 show single bands specific to class A and C *mec* gene complexes.

Previous use of antimicrobials may be associated with MRSP infection in dogs.

Furthermore, multiplex and duplex PCR assays for SCC*mec* typing revealed that 33 (63.4%) of 52 MRSP strains were type III-like, 18 (34.6%) belonged to type V, and only one was determined as nontypeable. The representative electrophoretic patterns of SCC*mec* type III-like (with fragments of both type 3 *ccr* and class A *mec*) and V (with fragments of both type 5 *ccr* and class C *mec*) are shown in Fig. 1.

To examine the presence of ET genes, amplification of the *expA* and *B* genes was conducted as shown in Fig. 2 (representative data). Table 2 shows the frequency of *expA* and *B* in the isolated *S. pseudintermedius* strains. The *expA*

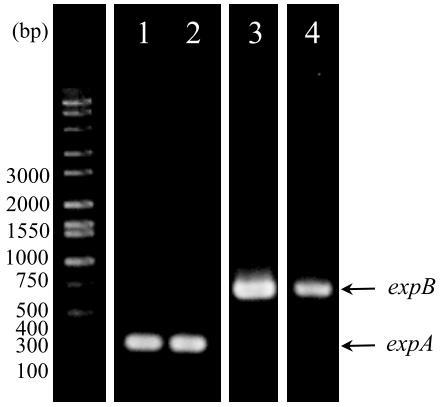

Fig. 2 PCR analysis of exfoliative genes.
Lane 1, a MSSP isolate carrying the *expA* gene; lane 2, positive control strain for *expA*; lane 3, a MSSP carrying the *expB* gene; lane 4, positive control strain for *expB*.

gene was detected at a rate of 22.7% (5/22) in methicillin-susceptible *S. pseudintermedius* (MSSP) strains in contrast to 1.9% (1/52) in MRSP strains; the latter MRSP strain was SCC*mec* type V. One MSSP isolate possessed the *expB* gene; however, the gene was not detected in any MRSP strain. There was no significant possession of ETs in *S. pseudintermedius* carrying the *mecA* gene, although they may be important virulence factors in canine pyoderma.

This study demonstrated that as many as 70.3% of 74 dogs diagnosed with superficial pyoderma had MRSP, implying the prevalence of MRSP in veterinary clinical practice in Japan. It is therefore important to rapidly, easily and feasibly determine *S. pseudintermedius* strains carrying the *mecA* gene and identify their genotypes not only to understand the epidemiological pattern but also for implementation of infection-control measures in veterinary clinical practice. To detect MRSP strains and their SCC*mec* types, we used several traditional PCR techniques that were complicated, cumbersome and time-consuming. We are currently designing improved multiplex PCR strategies.

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