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

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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 (SCCmec) 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 SCCmec 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; SCCmec; exfoliative toxin

Introduction

*Staphylococcus pseudintermedius* is a normal inhabitant of the skin and mucosae of dogs1,2. This species is also known to be the major pathogen of superficial pyoderma, one of the most common infectious diseases of canine cutaneous disorder3. Previous studies have revealed that *S. pseudintermedius* possess virulence factors such as exfoliative toxins (ETs) ExpA and B, which cause skin exfoliation4-6. However, few studies have described the distribution of ETs in *S. pseudintermedius* from canine superficial pyoderma4,5, 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 19997, MRSP infections have been increasing in small animal medicine8-12. According to previous studies, MRSP strains are mainly classified into two genotypes based on the type of staphylococcal cassette chromosome mec (SCCmec): SCCmec type III-like clones (informally designated type II-III by Descloix et al.13), which are found in Europe and many other areas across the world14-18, and type V clones, which are prevalent in North America, Korea and Thailand14,15,19-22. 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. We therefore conducted molecular analysis of MRSP strains isolated from canine superficial pyoderma and determined SCCmec 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 SCCmec 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. To identify methicillin resistance, a PCR method for detection of the meca gene was used. Subsequently, SCCmec typing of the MRSP strains identified was performed. To discriminate SCCmec types I to V, including type III-like, classified based on the ccr and mec gene complexes, two M-PCRs and one duplex PCR 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'-TTTATGACGCTATGCTCATT-3' (forward) and 5'-TCCTAAATTACCCGTCAAAAAAT-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. Molecular characteristics of the isolated S. pseudintermedius were investigated using several conventional PCR methods as previously described. 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, 29.3% in Korea and 47.8% in North China, and was similar to the previously reported 66.5% in Japan. 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.
Previous use of antimicrobials may be associated with MRSP infection in dogs.

Furthermore, multiplex and duplex PCR assays for SCCmec 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 SCCmec 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

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<tr>
<th>S. pseudintermedius</th>
<th>No. of strains (%)</th>
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<tr>
<td>Methicillin-susceptible</td>
<td>22 (29.7)</td>
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<tr>
<td>Methicillin-resistant</td>
<td>52 (70.3)</td>
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<tr>
<td>SCCmec-type III-like</td>
<td>33</td>
</tr>
<tr>
<td>SCCmec-type V</td>
<td>18</td>
</tr>
<tr>
<td>Nontypeable</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>S. pseudintermedius</th>
<th>n</th>
<th>No. of positive strains (%)</th>
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<tbody>
<tr>
<td>Methicillin-susceptible</td>
<td>22</td>
<td>5 (22.7)</td>
</tr>
<tr>
<td>Methicillin-resistant</td>
<td>52</td>
<td>1 (1.9)</td>
</tr>
</tbody>
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Fig. 1 Multiplex PCR analysis of ccr gene complex (A) and mec gene complex (B).
Lane 1, SCCmec type III-like MRSP; lane 2, SCCmec type V MRSP; lane 3, SCCmec 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.

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 SCCmec type V. One MSSP isolate possessed the \textit{expB} gene; however, the gene was not detected in any MRSP strain. There was no significant possession of ETs in \textit{S. pseudintermedius} carrying the \textit{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 \textit{S. pseudintermedius} strains carrying the \textit{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 SCCmec types, we used several traditional PCR techniques that were complicated, cumbersome and time-consuming. We are currently designing improved multiplex PCR strategies.

Acknowledgements

We are grateful to Dr. Koji Nishifuji (Tokyo University of Agriculture and Technology, Japan) for providing control strains for detection of exfoliative toxin genes. We also thank Ms. Akemi Suto and Dr. Reiko Usui for their help in collecting specimens.

References


