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 Koo1,*, Kuk Ju Ahn1, Suk-Kyung Lim2, Yong Ho Park1,*

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...
Fig. 1. Staphylococcal enterotoxins A (sea), B (seb), C (sec), D (sed), E (see), G (seg), H (she), 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 sea, tsst-1/see, seg, sei/seb, sed, and seh and uniplex PCR for sea were performed. Only a single isolate from a veterinary staff member (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 SECcanine 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 (SCCmec) typing of Staphylococcus intermedius group (SIG) isolates from Korean veterinary hospitals (veterinary staff, companion animal, and the veterinary hospital environment)

| Group/SCCmec 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  | 0 | 26 | 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.

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 SECcanine 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.
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 conclusion, eta, eth, 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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