Prevalence of *mecA* Gene and Antimicrobial Susceptibility in Staphylococci Taken from Dogs with Tumors With No Signs of Dermatitis and Healthy Dogs

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**ABSTRACT**

**Objectives**

The aim of the present study was to assess the prevalence of *mecA* gene and antimicrobial susceptibility in staphylococci taken from dogs with tumors but no signs of dermatitis and healthy dogs.

**Procedure**

Swabs were taken from the inguinal region of 72 healthy dogs referred to 2 private hospitals and 47 dogs referred to the teaching hospital at Azabu University that had presented with various tumors but had no other skin lesion.

**Results**

Of the 72 healthy samples taken, 10 isolates were identified as staphylococci and, within these isolates, 2 isolates were found to have the *mecA* gene. In the dogs with tumor, of the 47 samples taken, 16 isolates were identified as staphylococci and, within these, 8 isolates were found to have the *mecA* gene, so the number of dogs possessing *mecA* gene showed significantly higher when compared to healthy dogs. In 16 isolates from dogs with tumors, levels of susceptibility to ampicillin and amoxicillin were low. However, levels of susceptibility to cephalaxin, cefotaxime and amoxicillin/clavulanic acid were high, and there was no resistant strain.
against imipenem and vancomycin.

Conclusions
These data suggest that dogs with tumors could act as reservoirs of \textit{mecA} gene, if they show no skin lesions. Selection of effective antimicrobials is proposed as a treatment.

INTRODUCTION
Staphylococci is a well known agent of canine pyoderma and a major cause of surgical wound infections.$^{1-3}$ Coagulase positive isolates, in particular \textit{S pseudintermedius}, are recognized as the primary canine pathogen. Its ability to develop mechanisms that produce β-lactamase resistance to beta-lactam antimicrobials, and allow the \textit{mecA} gene to encode penicillin-binding protein 2a, which means that staphylococci has the potential to become resistance to a wide range of antimicrobials. The spread of \textit{mecA} positive staphylococci in animal hospitals and homes is problematic because methicillin-resistant strains may be transmitted not only among dogs but also between dogs and humans.$^{4}$ As the number of aged dogs has increased, new courses of chemotherapy and new techniques for treatment have been developed, and increasing numbers of dogs with tumors are being presented for treatment at animal hospitals. However, if non-tumor type skin lesions are not observed in these cases, then the prevalence of resistant staphylococci will often remain unconfirmed. In response to this, the current research aims to determine the extent to which dogs with tumors were also infected with staphylococci when compared to normal dogs, and further investigates the occurrence of \textit{mecA} gene in order to identify effective antimicrobial agents.

MATERIALS AND METHODS
Samples
Seventy two swabs were taken from the inguinal region of normal dogs referred to two private hospitals for vaccination or trimming. An additional 47 swabs were taken from the inguinal region of dogs referred to the teaching hospital at Azabu University that had presented with various tumors but had no other skin lesions.

Identification of Staphylococci
Swabs were immediately and directly inoculated on mannitol-salt agar and incubated at 37°C for 24 hr for elective isolation of staphylococci. The bacteria that were grown in mannitol-salt agar were then pure cultured at 37°C for 24 hr on Columbia agar supplemented with 5% sheep blood in aerobic conditions. The isolates were identified as gram-positive cocci with negative oxidase activity and positive catalase activity. Coagulase tests were also performed to identify these isolates as positive or negative. The biochemical profiles of isolates were tested using API ID32 STAPH® (SYSMEX bio-Mérieux Co., Ltd) identification system.

\textbf{Figure 1} Discrimination of the isolates by PCR using the standard genomic DNA isolated from \textit{S. pseudintermedius} strain, NVAU02008 and \textit{S. intermedius} strain, P•4A. PCR products of strains normal dog Nos.1-7 (lanes 1-7), are shown; M; marker, PC; positive control.
Antimicrobial Susceptibility
Antimicrobial susceptibility was investigated using the disc diffusion method, according to Clinical and Laboratory Standard Institute guidelines. The following antimicrobial agents were included in this study: ampicillin, amoxicillin, amoxicillin/clavulanic acid, cephalexin, cefotaxime, imipenem, fosfomycin, vancomycin, ofloxacin, enrofloxacin, erythromycin, clindamycin, gentamicin, chloramphenicol, doxycycline, and trimethoprim/sulfamethoxazole. The isolates were classified as susceptible, intermediate or resistant. All discs used in this test were made by Nissui Co., Ltd, and Mueller-Hinton agar was used.

Isolation of Genomic DNA
The template DNA for polymerase chain reaction (PCR) amplification was purified with modifications according to the method by Hartmann et al (2005).2 Genomic DNA from staphylococci was isolated using GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, St. Louis, MO), following the manufacturer’s procedure.

Identification of \( S.\) pseudintermedius
The commercial kit is not able to identify \( S.\) pseudintermedius, that is instead misidentified as \( S.\) intermedius. Therefore, in order to discriminate \( S.\) pseudintermedius isolate from \( S.\) intermedius isolate, recently described PCR of the staphylococcal thermoneclease gene (nuc) was perfomed.5 As the standard, genomic DNA was isolated from \( S.\) pseudintermedius strain, NVAU02008 and \( S.\) intermedius strain, P•4A by the method of Hartmann et al.1 Oligonuceotide primer sequences of the sense (pse-F2) and reverse primer (pse-F5) for \( S.\) pseudintermedius species were 5'-TRGGCAGTAGGATTCCGTA-3' and 5'-CTTTGTTGCTYCMMTTGG-3', respectively.6 Primer sequences of the sense (in-F) and reverse (in-R3) for \( S.\) intermedius species were 5'-CATGTCATATTCTATTGCGAATGA-3' and 5'-AGGACCATCACCATTGACATATGAAACC-3', respectively.6 The reaction mixture for the PCR consisted of 2 μl of DNA in a total volume of 50 μl composed of 2.5 units of Ex Taq DNA polymerase (Takara Bio Inc., Shiga Japan), 0.4μM of each primer, 1x Ex Taq buffer and 0.2 mM each dNTP. Reaction mixtures were thermally cycled initially at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 35 sec, and extension at 72°C for 1 min and then final extension at 72°C for 2 min. PCR products were analyzed on 2% agarose gel containing 0.5μg/ml ethidium bromide (Sigma-Aldrich), and formation of 926- and 430-bp DNA bands was considered to be a positive results for infection of \( S.\) pseudintermedius and \( S.\) intermedius, respectively.

Identification of \( mecA\) gene
Isolates were tested for the presence of the \( mecA\) gene according to the PCR method.

Figure 2 2% agarose gel electrophoretic profile of PCR products from genomic DNA extracted from staphylococci. PCR positive products of Strains Nos.1-8 (lanes 1-8), PCR negative products of Strains Nos.9-16 (lanes 9-16) are shown. M, 100 bp DNA ladder marker; NC, negative control.
described by Jonas et al.7 PCR was performed for the detection of mecA gene DNA derived from isolates. The sense and reverse primers for mecA gene (GenBank accession No. EF692632) were mecA1 and mecA2, respectively. The sequences of the mecA1 primer and the mecA2 primer were 5’-GTAGAAATGACTGAAGTCCGATAA-3’ and 5’-CCAATTCCACATTGTTTCGGTCTAA-3’, respectively. Standard PCR was performed. PCR products were electrophoresed on 2% agarose gel, and the formation of a 310-bp DNA band was considered to be a positive result for mecA gene amplification.

### Statistical Analysis

Statistical analysis was calculated for the number of dogs possessing mecA between normal dogs and dogs with tumors, between the duration of treatment of mecA gene positive strains and negative strains in dogs with tumors, and between age of treatment of mecA gene positive strains and negative strains in dogs with tumors, by Mann-Whitney U-test. In this test p<0.05 was considered to indicate a significant difference.

### RESULTS

Ten isolates of 72 swabs taken from normal dogs, and 16 isolates of 47 swabs taken from dogs with tumors, were identified as staphylococci due to gram-positive cocci with negative oxidase activity and positive catalase activity.

Of the 10 isolates from normal dogs, 7 isolates (Nos.1-7) were classified as S. pseudintermedius by PCR (Fig.1) and 2 isolates (Nos. 8,9) were classified as S. simulans and 1 isolate (No.10) was classified as S. xylosus by the commercial kit. Furthermore, genomic DNA isolated from 10 isolates from normal dogs was assayed by specific PCR for mecA gene and formation of 310 bp DNA band was considered to be infection positive by two isolates (Nos.1 and 2).

Table 1 shows the susceptibility of the 10 isolates obtained from normal dogs to the various antimicrobials; levels of susceptibility to ampicillin and amoxicillin were only 30% and 50% respectively. However, levels of susceptibility to cephalexin, cefotaxime, and amoxicillin/clavulanic acid were higher, and the susceptibility rates were 80, 90, or 100%, respectively. Genomic DNA isolated from 10 isolates from normal dogs was assayed by specific PCR for mecA gene and formation of 310 bp DNA band was considered to be infection positive by two isolates (Nos.1 and 2).

Of the 16 isolates from dogs with tumors, 15 isolates (Nos.1-15) were classified as S. pseudintermedius by PCR and 1 isolate (No.16) was classified as S. haemolytica by the commercial kit.

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| S%  | 30  | 50  | 50  | 60  | 70  | 70  | 80  | 80  | 90  | 90  | 100 | 100 | 100 | 100 | 100 | -    |

**Table 1** Antimicrobial resistance and susceptibility patterns for staphylococci isolates from 10 healthy dogs


S: Susceptible, I: Intermediate, R: Resistant
DNA isolated from 16 isolates was assayed by specific PCR for meca gene and formation of 310 bp DNA band was considered to be infection positive by 8 isolates (Fig.2).

There was a significant difference in the number of dogs possessing meca between dogs with tumors and normal dogs, namely 8/47 and 2/72 (p=0.031).

Table 2 shows the susceptibility of the 16 isolates obtained from dogs with tumors to the various antimicrobials; levels of susceptibility to ampicillin and amoxicillin were only 31% and 44% respectively. Ampicillin therefore had no effect against meca positive staphylococci. However, levels of susceptibility to cefalexin, cefotaxime and amoxicillin/clavulanic acid were higher, and the susceptibility rates were 75, 81, 81%, respectively. All of the strains were susceptible against imipenem and vancomycin.

Diagnoses for the dogs with meca positive were: mucocele of salivary gland plus mast cell tumor, hemangiopericytoma, squamous cell carcinoma, multicentric lymphoma, well differentiated fibrosarcoma plus mast cell tumor, hemangiopericytoma, anal sac apocrine gland adenocarcinoma, and adenocarcinoma. The dogs with meca negative were diagnosed as: rectum adenocarcinoma, hepatocellular carcinoma, hemangiopericytoma, mammary carcinoma, multicentric lymphoma, multilobular osteochondrosarcoma, oral melanoma, and undifferentiated tumor (Table 3). There was no tendency for cases of a particular diagnosis to be concentrated as either meca positive or meca negative. Duration of treatment and age between both meca gene positive strains (Nos.1-8) and negative strains (Nos.9-16) did not show statistical differences, with p=0.194 and p=0.72, respectively.

**DISCUSSION**

The meca gene is methicillin-resistant gene and gene transfer may occur to S aureus...
and/or *S. pseudintermedius*. The mecA gene is contained in staphylococcal cassette chromosome elements and is transmitted among Staphylococci, and therefore resistant Staphylococci which gained this gene could transmit not only among dogs but also between dogs and humans.

In dogs, a major agent of skin lesion is *S. pseudintermedius,* and in the present study, all of coagulase positive isolates were identified as *S. pseudintermedius,* with *S. aureus* not isolated. However it has been reported that *S. pseudintermedius* has also been isolated from humans* and therefore *S. pseudintermedius* with mecA gene is a problematic concern for not only veterinary hospitals, but also for public health.

In this report, the mecA gene was identified more commonly in staphylococci isolated from dogs with tumors but no skin lesions than in normal dogs. As most cases had received treatment for tumors over an inevitably long period, it is possible that these dogs might have become compromised hosts to obtain this resistant gene, and might act as a reservoir for humans. In regards to antimicrobial susceptibility, cefalexin, and amoxicillin/clavulanic acid have been demonstrated to be effective as antimicrobials and these data were higher than the results for canine pyoderma shown by Kawakami et al*).

**CONCLUSIONS**

In order to control the prevalence of mecA gene positive staphylococci, proper selection of antimicrobiotics, and the enforcement of hygiene management including cleaning in the hospital and washing hands is required for small animal clinic and public health.
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