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

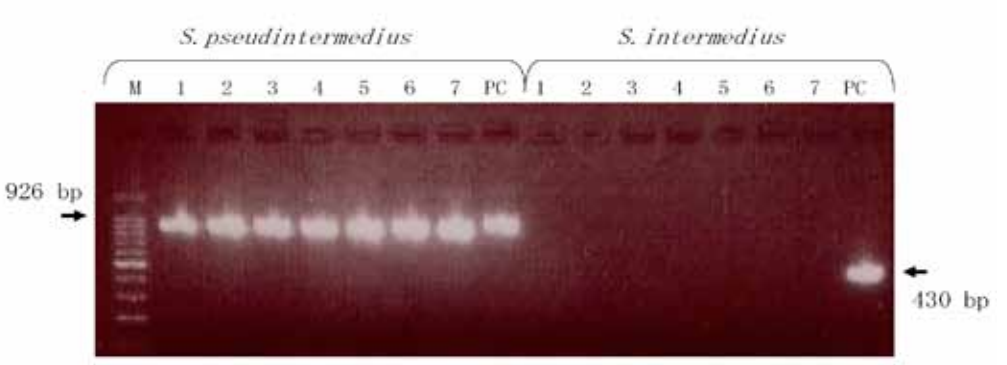
Procedure

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 cephalexin, cefotaxime and amoxicillin/clavulanic acid were high, and there was no resistant strain

Figure 1 *Discrimination of the isolates by PCR using the standard genomic DNA isolated from S. pseudintermedius strain, NVAU02008 and S. intermedius strain, P•4A. PCR products of strains normal dog Nos.1-7 (lanes 1-7), are shown; M; marker, PC; positive control.*



against imipenem and vancomycin.

Conclusions

These data suggest that dogs with tumors could act as reservoirs of *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 *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 *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 *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 *mecA* gene in order to identify effective antimicrobial agents.

MATERIALS AND METHODS

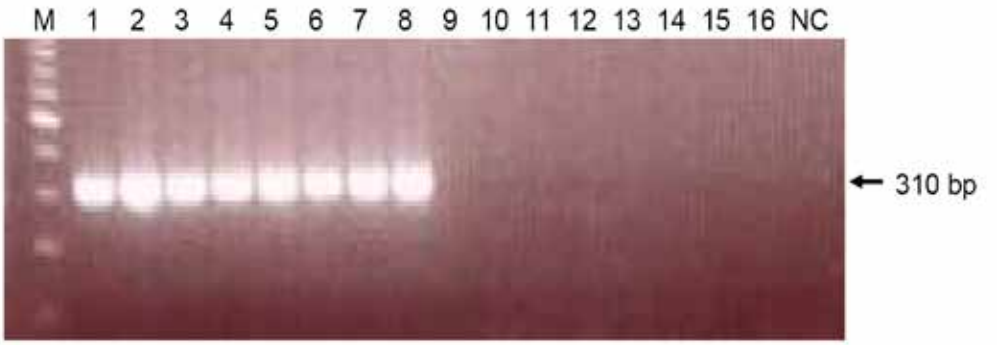
Samples

Seventy two swabs were taken from the inguinal region of normal dogs referred to two private hospitals for vaccination or hair 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 bioMérieux Co., Ltd) identification system.

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.*



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 thermonuclease 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'-TRGGCAGTAGGATTCGTTAA-3' and 5'-CTTTTGTGCTYCMTTTTGG-3', respectively.6 Primer sequences of the sense (in-F) and reverse (in-R3) for *S. intermedius* species were 5'-CATGTCATATTATTGCGAATGA-3' and 5'-AGGACCATCACCATTGACATATTGAAACC-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

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

No.	AB	AM	EM	ER	OF	ST	CL	CE	GM	CP	CT	DO	CV	FO	IP	VC	*mecA*
1	R	R	R	I	R	R	I	I	R	I	S	S	S	S	S	S	+
2	R	R	S	R	R	S	S	S	R	S	S	S	S	S	S	S	+
3	S	S	R	S	S	S	R	I	S	S	I	S	S	S	S	S	-
4	R	I	S	S	S	I	S	S	S	S	S	I	S	S	S	S	-
5	R	S	I	I	S	S	S	S	S	S	S	S	S	S	S	S	-
6	R	I	S	S	S	I	S	S	S	S	S	S	S	S	S	S	-
7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-
8	R	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	-
9	R	I	S	S	S	S	I	S	S	S	S	S	S	S	S	S	-
10	S	S	R	I	R	S	S	S	S	S	S	S	S	S	S	S	-
S%	30	50	50	60	60	70	70	80	80	90	90	90	100	100	100	100	

AB: ampicillin, AM: amoxicillin, EM: erythromycin, ER:enrofloxacin, OF: ofloxacin, ST: sulfamethoxazole+trimethoprim, CL: clindamycin, CE:cephalexin, GM: gentamicin, CP: chloramphenicol, CT: cefotaxime, DO: doxycycline, CV:clavulanic acid+amoxicillin, FO: fosfomycin, IP: imipenem, VC: vancomycin
S: Susceptible, I: Intermediate, R: Resistant

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 *mecA* 1 and *mecA* 2, respectively. The sequences of the *mecA* 1 primer and the *mecA* 2 primer were 5'-GTAGAAATGACTGAACGTCCGATAA-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. 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.1and 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. haemolytics by the commercial kit. Furthermore, genomic

Table 2 *Antimicrobial resistance and susceptibility patterns for staphylococci isolates from 16 dogs with cancer*

No.	AB	AM	EM	ER	OF	ST	CL	CE	GM	CP	CT	DO	CV	FO	IP	VC	*mecA*
1	R	I	R	R	R	R	S	S	R	S	S	R	R	S	S	S	+
2	R	R	R	R	S	S	S	S	S	S	I	S	S	S	S	S	+
3	R	R	I	R	R	I	R	R	S	S	S	S	S	S	S	S	+
4	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	+
5	R	R	R	R	R	I	R	R	S	S	S	S	S	S	S	S	+
6	R	R	S	R	R	S	R	R	S	R	R	S	S	S	S	S	+
7	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	+
8	R	S	R	S	S	S	R	S	S	S	S	S	S	I	S	S	+
9	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	-
10	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-
11	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-
12	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-
13	R	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	-
14	S	S	S	S	S	I	S	S	R	S	S	S	S	S	S	S	-
15	S	I	S	S	S	S	S	S	S	S	I	S	S	S	S	S	-
16	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	-
S%	31	38	44	56	63	63	63	69	75	75	75	81	81	94	100	100	

S%: Percentage of susceptible, AB: ampicillin, ST: sulfamethoxazole+trimethoprim, AM: amoxicillin, GM: gentamicin, OF: ofloxacin, ER:enrofloxacin, EM: erythromycin, CL: clindamycin, CE:cephalexin, CP: chloramphenicol, DO: doxycycline, CV:clavulanic acid+amoxicillin, CT: cefotaxime, FO: fosfomycin, IP: imipenem, VC: vancomycin
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 cephalexin, 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

Table 3 *Age, duration from reference, diagnosis and treatment of dogs with tumor with staphylococci*

No	Age	Du*	Diagnosis	An*	Ra*	Ca*	Op*
1	14Y6M	71M	mucocele of salivary gland, mast cell tumor	○	-	-	○
2	12Y8M	38M	hemangiopericytoma	-	○	-	○
3	12Y	35M	squamous cell carcinoma	-	-	○	○
4	10Y2M	15M	multicentric lymphoma	○	○	○	-
5	11Y3M	13M	well differentiated fibrosarcoma + mast cell tumor	○	○	-	○
6	9Y10M	2M	hemangiopericytoma	○	-	-	○
7	9Y11M	2M	anal sac apocrine gland adenocarcinoma	○	-	○	○
8	11Y8M	0.07M	adenocarcinoma	-	-	-	-
9	9Y8M	27M	rectum adenocarcinoma	○	-	-	○
10	12Y5M	10M	hepatocellular carcinoma	○	-	-	○
11	12Y9M	7M	hemangiopericytoma	○	-	-	○
12	14Y2M	5M	mammary carcinoma	-	-	-	○
13	11Y1M	3M	multicentric lymphoma	-	-	○	-
14	9Y6M	0.5M	multilobular osteochondrosarcoma	-	-	-	-
15	11Y9M	0.25M	oral melanoma	-	-	-	-
16	12Y4M	0M	undifferentiated tumor	-	-	-	-

Du; Duration of treatment, An; Antibiotics, Ra; Radiotherapy, Ca; Carcinostatics, Op; Operation

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*,[8] 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[9] and therefore *S. pseudintermedius* with *mecA* gene is a problematic concern for not for 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[4]).

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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