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Comparison of Harboring the Resistance Gene and Disc Diffusion Susceptibility Test Result in *Staphylococcus pseudintermedius* from the Bacterial Dermatitis

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Abstract : Bacterial dermatitis is common disease that is necessary to treat with antibiotics. In recent, antibiotic-resistant bacteria is being increased in worldwide. The purpose of the present study was to evaluate the prevalence of resistant genes in *Staphylococcus (S.) pseudintermedius* isolated from dogs, and to compare the resistant gene profile with the result of antibiotic disc diffusion test. A total of seven *S. pseudintermedius* was included in the study. Bacterial identification was performed by 16S ribosomal RNA gene sequence analysis. *S. pseudintermedius* isolates had more than one antibiotic resistant gene (*mec*A, *bla*Z and *aac*(*6'*)/*aph*(*2''*). While all isolates were PCR positive to *bla*Z gene, only two isolates were resistant to amoxicillin/clavulanate. Among five isolates harboring gentamicin resistance, one isolate was negative to *aac*(*6'*)/*aph*(*2''*)-targeted PCR. Taken together, the results suggest that resistant gene-targeted PCR and disc diffusion test are complementary to detect antibiotic resistance.

Key words **:** antibiotic susceptibility, bacterial dermatitis, resistance gene, dog.

Introduction

Staphylococcus (*S.*) *pseudintermedius* is a normal inhabitant of the skin and mucosa and can be isolated from the nares, mouth, pharynx, forehead, groin, and anus of healthy dogs (10). The anal region and the nose are colonized more frequently than other areas in healthy dogs. In general, knowledge of the pathogenesis of *S. pseudintermedius* is limited; however, approximately 80% of canine pyoderma is caused by the infection of *S. pseudintermedius* (8). In addition, several studies have reported the detection of methicillin-resistant *S. pseudintermedius* with same staphylococcal cassette chromosome *mec* (SCC*mec*) type among hospitalized animals and veterinary staff, suggesting the zoonotic potential of the bacteria and its resistant gene transfer (6,7,16).

Since the commercial introduction of antibiotics into clinical uses, staphylococci have shown rapid acquisition of resistance to commonly used antibiotics, particularly in those strains associated with nosocomial infections (14,15,18). In the development and spread of the resistance, plasmids are likely to play an important role by acting as carriers of resistance genes or as vectors for transposon-borne resistance genes (5). For aminoglycosides, the most widespread mechanism of resistance is the modification of the drug by plasmid- or transposon-encoded aminoglycoside-modifying enzymes (AMEs) (11,12). They can be divided into three classes: aminoglycoside acetyltransferases (AACs), aminoglycoside phosphotransferases (APHs), and aminoglycoside nucleotidyltransferases (ANTs) (2). Among them, the *aac(6')/aph(2'')* gene is the most frequently encountered in aminoglycoside-resistant *S. aureus* or *S. pseudintermedius* (2,9,17).

In some circumstances, disc diffusion test for antibiotic resistance can make a false-negative result (e.g., inducible clindamycin resistance usually produces false-negative result in conventional disc diffusion tests) (3). In this study, we examined antibiotic resistance profiles of the *S. pseudintermedius* isolates from canine pyoderma in South Korea by using standard disc diffusion test and resistant gene-targeted PCR and then compared the results between two test methods.

Materials and Methods

Isolation and identification of S. pseudintermedius

A total of 56 canine patients with bacterial dermatitis was included in the study. The sample was collected by cotton swab on the skin lesions. After the sampling, the swab samples were cultured on Trypticase Soy Agar with 5% sheep blood at 37ºC for 24-48 h. The primary identification of *Staphylococcus* spp. was made based on the colony morphology, complete or incomplete hemolysis, and Gram staining. To identify *S. pseudintermedius*, extracted bacterial genomic DNA was amplified using a pair of 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') for the 16S ribosomal RNA gene (16S rDNA). The PCR was performed an initial denaturation at 94ºC for 2 min was followed by 35 cycles at 94ºC for 1 min, 57ºC for 1 min, and 72ºC for 70 s. A final run at 72ºC for 3 min com-

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Table 1. Primers for identification of bacterial antibiotic resistance gene

Target gene	Primer name	Neucleotide sequence (5' → 3')	Amplicon size
*mec*A	OX1	AAAATCGATGGTAAAGGTTGGC	553
	OX2	AGTTCTGCAGTACCGGATTTGC	
blaZ	BZ1	ACTTCAACACCTGCTGCTTTC	173
	BZ2	TGACCACTTTTATCAGCAACC	
aac(6')/aph(2")	GN1	CCAAGAGCAATAGGGCATACC	222
	GN2	CACACTATCATAACCACTACCG	

pleted the program. All amplicons were purified and sent for DNA nucleotide sequence analysis (SolGent, Daejeon, Korea). The sequence homology of deduced nucleotide sequences was analyzed with the BLAST search program (National Center for Biotechnology Information, NCBI) based on the interpretative criteria for identifying bacteria and fungi by DNA target sequence (The Clinical and Laboratory Standards Institute guideline MM-18A, CLSI, Wayne, PA, USA).

Antibiotic susceptibility testing

To examine the antibiotic resistance of the isolated *S. pseudintermedius*, antibiotic susceptibility testing was performed by the Kirby-Bauer disc diffusion test. For the test, 3 antibiotics were selected: oxacillin, gentamicin, and amoxicillin/clavulanate (BBL™ Sensi-Disc™ Susceptibility test disc, BD, NJ, USA). The interpretation of the test results were made based on the CLSI guideline for veterinary antimicrobial susceptibility testing for pathogens of animal origin (CLSI, 2008).

Antibiotic resistance gene

To examine the presence of antibiotic-resistant genes, PCR was performed for *mec*A, *blaZ*, and *aac(6')/aph(2")* genes according to the previously described protocols (Table 1). The thermal cycling was set at 35 cycles, with 30 sec of denaturation at 94°C, 30 sec of annealing at 55°C, and 60 sec of extension at 72°C. Three minute of premelting at 94°C, and 3 min of final extension at 72°C, was also performed. All PCR products were identified with agarose gel electrophoresis and were verified with sequencing.

Table 2. Comparison of antibiotic disc diffusion and resistant gene expression in *Staphylococcus pseudintermedius* isolated from bacterial dermatitis

ID	Disc diffusion			PCR		
	OX	G	AmC (mm)	*mec*A	*blaZ*	*aac(6')/ aph(2")*
1	R	R	S	-	+	+
2	R	R	S	-	+	+
3	R	R	R	+	+	-
4	R	R	S	-	+	+
5	R	R	S	-	+	+
6	S	S	R	-	+	-
7	R	S	S	-	+	-

OX, oxacillin; G, gentamicin; AmC, Amoxicillin-clavulanic acid; R, resistant; S, susceptible; -, negative; +, positive

Results

Among the bacterial samples (n = 56), seven *S. pseudintermedius* were isolated and used in the study. Disc diffusion test showed that all isolates had resistance to more than one antibiotic (Table 2). Among them, oxacillin- (86%) and gentamicin resistance (71%) were most common. Antibiotic resistant gene-targeted PCR showed *mec*A (n = 1), *blaZ* (n = 7), *aac(6')/aph(2")* (n = 4) gene positive results. Concordance rate between PCR and antibiotic disc diffusion was 86%. Among six oxacillin-resistant isolates, only one isolate was positive to *mec*A gene while all of them had *blaZ* gene concurrently. Of five gentamicin-resistant isolates, one isolate did not show positive result in *aac(6')/aph(2")* gene-specific PCR. All isolates were *blaZ* gene positive, but only two isolates were resistant to amoxicillin/clavulanate.

Discussion

Antibiotic disc diffusion test can result in the erroneous result depending on the temperature, condition of medium, and improper incubation time (4). It can produce false negative results in antibiotic susceptibility testing, resulting in the usage of improper antibiotics to the patient with resistant bacterial infection. In particular, it is reported that some of staphylococci reveal susceptible results to an antibiotic *in vitro* susceptibility testing, however, they actually produce resistance to the corresponding antibiotic by inducible resistant mechanisms (3). This phenomenon suggests the necessity of complementary test protocol for the conventional disc diffusion test. In this study, the isolated staphylococci were susceptible to amoxicillin/clavulanate, whereas all of them have *blaZ* gene, suggesting the possibility of inducible resistance of the bacteria if the antibiotic is used for the patient.

Penicillin resistance is produced by staphylococcal β-lactamase production, and the modification of penicillin binding protein (PBP) encoded by *mec*A gene. Staphylococcal β-lactamase production is mediated by *blaZ* gene (13). Increase of inhibition zone of amoxicillin/clavulanate disc suggests β-lactamase overproduction (1). In case of oxacillin-resistant, all of *mec*A was negative but *blaZ* was positive, indicating the possibility of presence of another mechanism for oxacillin resistance. This result suggests that methicillin-susceptible and -resistant strains are not distinguishable only by

resistant gene-targeted PCR and disc diffusion test should be performed in company with the disc diffusion test. Taken together, this study suggests that resistant gene-targeted PCR test and disc diffusion test are complementary to detect antibiotic resistance in veterinary diagnostic laboratories.

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세균성 피부염 개에서 분리된 *Staphylococcus pseudintermedius* 에서 항생제 감수성 검사와 내성 유전자 획득의 비교

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요 약 : 세균성 피부염은 항생제 치료가 필요한 흔한 질환이다. 최근 항생제 내성 세균이 전 세계적으로 증가하고 있는 추세이다. 본 연구에서는 개 피부염 병변에서 분리한 *S. pseudintermedius*의 항생제 내성 유전자 발현을 조사하고, 이를 디스크 감수성 시험결과와 비교하였다. 총 7개의 *S. pseudintermedius* 균주가 연구에 포함되었다. 세균 동정은 16S rDNA 염기 서열 분석으로 실시하였다. 조사 결과 분리한 *S. pseudintermedius* 모두가 1개 이상의 항생제 내성 유전자(*mec*A, *bla*Z and *aac*[*6′*]/*aph*[*2″*])를 보유하였다. 전체 분리균주가 *bla*Z 유전자에 양성을 보였으나, 두 개 균주만이 amoxicillin/clavulanate에 내성을 나타냈다. Gentamicin 내성을 나타낸 5개 균주 중, 1개는 *aac*(*6′*)/*aph*(*2″*) 유전자에 대한 PCR 검사에서 음성을 나타냈다. 이 결과는 내성 유전자 검출 PCR 기법과 디스크 감수성 시험 기법이 항생제 내성을 검사하는 데에 있어 상호보완적인 관계를 가짐을 제시한다.

주요어 : 항생제감수성, 세균성 피부염, 내성유전자, 개