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Antibiotic Resistance Profiles of *Staphylococcus pseudintermedius* Isolates from Canine Patients in Korea

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In this study, the prevalence of antibiotic resistance was examined among 74 *Staphylococcus pseudintermedius* strains recently isolated from clinical cases of canine pyoderma and otitis externa at the veterinary teaching hospital at Konkuk University, Korea. Bacterial resistance to the nine commonly used antibiotics was evaluated by a standard disk diffusion technique based on the guidelines of the Clinical and Laboratory Standards Institute. The results demonstrated that most *S. pseudintermedius* isolates were resistant to penicillin (95.9%) or tetracycline (91.9%), but highly susceptible to amoxicillin/clavulanic acid (90.5%). Among the 74 isolates, 13 *mecA*-positive and methicillin-resistant *S. pseudintermedius* (MRSP) strains were identified, displaying a high level of resistance (84.6–100%) to each of the individual antibiotics evaluated, with the exception of amoxicillin/clavulanic acid (46.2% resistance). Notably, all of the MRSP isolates exhibited simultaneous resistance to four or more different antibiotics, indicating that they are multiple drug resistant (MDR) strains. Taken together, these results imply that more careful selection or prescription of antibiotics for canine pyoderma and otitis externa should be required for reducing the emergence and/or spread of MDR strains, especially MDR–MRSP isolates, in veterinary pet clinics in Korea.

Keywords: Antibiogram, canine isolates, *S. pseudintermedius*, pyoderma, Korea

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Staphylococcus pseudintermedius (formerly referred to as *S. intermedius*) is an important opportunistic pathogen frequently associated with various clinical infections in dogs, such as canine pyoderma and otitis externa [9]. A recent study reported that this microorganism has been responsible for more than 90% of the clinical cases of canine pyoderma [22]. To date, a therapeutic strategy for canine pyoderma caused by *S. pseudintermedius* is based on either systemic or topical applications of antibiotics. However, increasing evidence suggests that prolonged antibiotic treatment [12, 22] and/or incorrect selection of antibiotics may increase the opportunity for production of certain populations of multiple-drug-resistant *S. pseudintermedius* (MDRSP) strains [16].

Recently, MDRSP or methicillin-resistant *S. pseudintermedius* (MRSP) has emerged as a serious problem in veterinary practice [6, 10, 12]. Since it has been reported that zoonotic transfer of *S. pseudintermedius* from dogs to humans is possible [2, 5, 8, 23], the potential carriage of MDRSP and MRSP in pet dogs can be a critical public health problem. Currently, limited information on the prevalence of MDRSP and MRSP is available in veterinary practice in Korea. In this study, therefore, we investigated the prevalence of antibiotic resistance among a total of 74 clinical isolates of *S. pseudintermedius* recently collected from canine patients suffering from pyoderma and otitis externa at the veterinary teaching hospital at Konkuk University during the years 2006–2008.

Materials and Methods

Isolation and Identification of *S. pseudintermedius*

A total of 74 *S. pseudintermedius* strains were isolated from the clinical cases of canine pyoderma and otitis externa submitted to the veterinary teaching hospital at Konkuk University (Seoul, Korea)

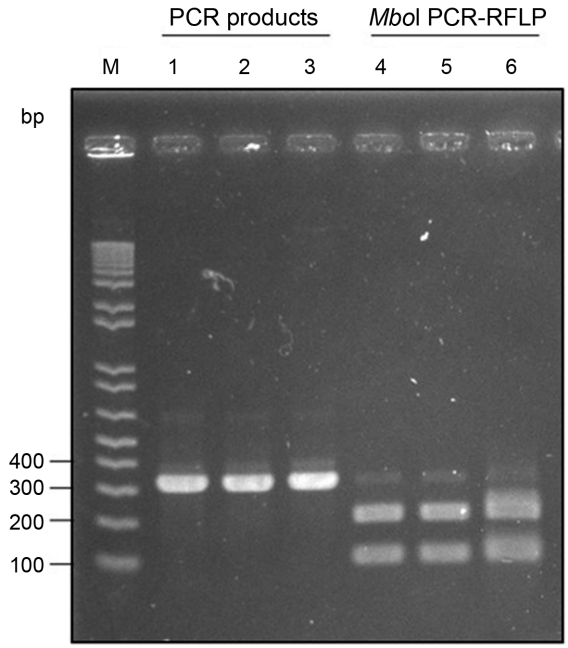

Fig. 1. Molecular identification of *S. pseudintermedius* by a recently described PCR–RFLP analysis.
Lane M, 1-kb Plus DNA Ladder (Invitrogen); 1 to 3, the expected 320-bp PCR amplicon of the *pta* genes from genomic DNA extracted from three putative *S. pseudintermedius* isolates; 4 to 6, *Mbo*I RFLP patterns of the PCR products (lanes 1 to 3) with characteristics typical of *S. pseudintermedius*.

during the period 2006 to 2008. All of the staphylococcal isolates were preliminarily screened by standard microbiological procedures including Gram staining, hemolysis, catalase test, and production of coagulase and DNase. Isolates were further differentiated as *S. pseudintermedius* by the polymerase chain reaction (PCR) using species-specific primers for the nuclease (*nuc*) gene as previously described [4], as well as a recently developed PCR–restriction fragment length polymorphism (RFLP) method [1], as shown in Fig. 1.

Determination of Antibiotic Resistance

For phenotypic detection of antibiotic resistance, antimicrobial susceptibility was determined by a Kirby–Bauer disk diffusion test with commercially obtained disks (BBL Sensi-Disc Susceptibility Test Discs; Becton Dickinson, MD, USA) for aerobic bacteria. The 74 *S. pseudintermedius* isolates in this study were evaluated for resistance to the nine commonly used antibiotics, penicillin (10 units), tetracycline (30 μg), sulfamethoxazole/trimethoprim (1.25/23.75 μg), erythromycin (15 μg), clindamycin (2 μg), gentamycin (10 μg), oxacillin (1 μg), chloramphenicol (30 μg), and amoxicillin/clavulanic acid (20/10 μg). In general, these antibiotics are known to be commonly used in veterinary medicine not only for canine pyoderma but also for a variety of animal infectious diseases in Korea as well as worldwide. Interpretation of antibiotic susceptibility was carried out by measuring the diameter of the growth inhibition zone according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [17].

PCR Detection of Methicillin or Penicillin Resistance Genes

Genotypic determination of antibiotic resistance was performed by detecting both *mecA* and *blaZ* genes, which encode penicillin binding protein 2a (conferring methicillin resistance) and β-lactamase (conferring penicillin resistance), respectively. PCR conditions were as described by El Zubeir *et al*. [4]. The identity of amplified PCR products was confirmed by direct DNA sequencing at Macrogen (Korea).

RESULTS

During the study period 2006–2008, a total of 124 staphylococcal strains were isolated from skin or ear lesions of 121 canine patients (data not shown). Among those strains, 74 were finally confirmed to be *S. pseudintermedius* by both biochemical and molecular genetic identification methods (see Materials and Methods). All of the *S. pseudintermedius* strains represented single isolates from individual canine patients.

The antibiotic susceptibility of all 74 *S. pseudintermedius* isolates is summarized in Table 1. The highest antibiotic resistance was observed toward penicillin (71 isolates; 95.9% resistance), tetracycline (68; 91.9%), and sulfamethoxazole/trimethoprim (49; 66.2%), whereas the lowest resistance was observed toward amoxicillin/clavulanic acid (7; 9.5%),

Table 1. Antimicrobial susceptibility of 74 *S. pseudintermedius* isolates from canine pyoderma and otitis patients.

Antimicrobial agent	Number of *S. pseudintermedius* isolates (%)		
	Susceptible	Intermediate	Resistant
Amoxicillin/Clavulanic acid	67 (90.5)	0 (0.0)	7 (9.5)
Oxacillin	55 (74.3)	6 (8.1)	13 (17.6)
Chlorampenicol	42 (56.8)	5 (6.8)	27 (36.5)
Gentamycin	25 (33.5)	3 (4.1)	46 (62.2)
Clindamycin	18 (24.3)	10 (13.5)	46 (62.2)
Erythromycin	14 (18.9)	15 (20.3)	45 (60.8)
Sulfamethoxazole/Trimethoprim	13 (17.6)	12 (16.2)	49 (66.2)
Teteracycline	6 (8.1)	0 (0.0)	68 (91.9)
Penicillin	3 (4.1)	0 (0.0)	71 (95.9)

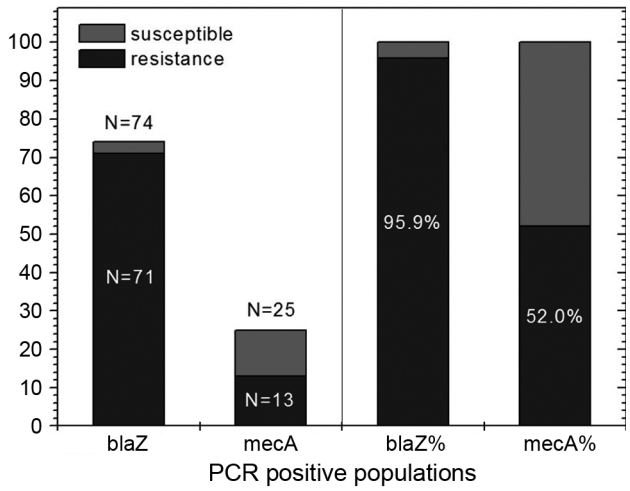

Fig. 2. Correlation between the presence of *mecA* and *blaZ* and bacterial resistance to oxacillin and penicillin among the 74 *S. pseudintermedius* isolates from canine patients.

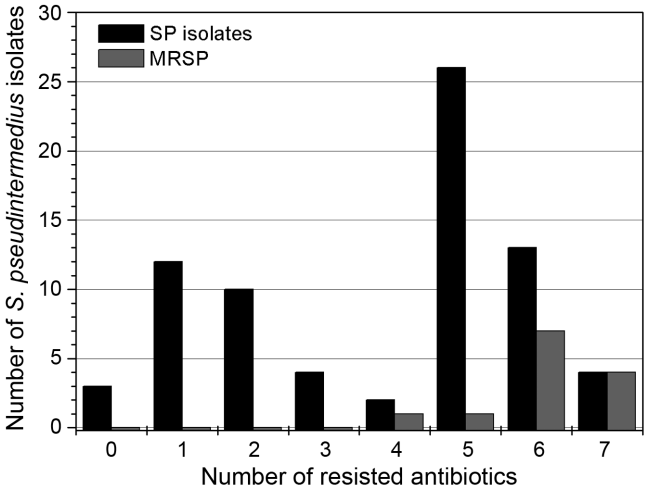

Fig. 3. Prevalence of multiple drug resistance (MDR) among the 74 *S. pseudintermedius* isolates from canine patients, including methicillin-resistant *S. pseudintermedius* (MRSP).

oxacillin (13; 17.6%), and chlorampenicol (27; 36.5%) (Table 1).

PCR detection of both *blaZ* and *mecA* demonstrated that almost all of the *blaZ*-positive isolates (95.9%) were resistant to penicillin, with the exception of three (4.1%), suggesting the existence of a subpopulation of *blaZ*-positive penicillin-susceptible isolates (Fig. 2). In addition, all 13 of the oxacillin-resistant isolates tested positive for *mecA* by PCR, indicating that they were *mecA*-mediated MRSP isolates (Fig. 2). Not surprisingly, all of these isolates displayed a high level of resistance (>84.6%) to each of the individual antibiotics evaluated in this study, with the exception of amoxicillin/clavulanic acid (46.2% resistance) (Table 2).

Antibiotic resistance profiling demonstrated that 58 of the 74 *S. pseudintermedius* isolates (78.4%) possessed a multiple drug resistance (MDR) phenotype, exhibiting simultaneous resistance to four or more different classes of antibiotics other than β-lactams (Fig. 3). It should be noted that all 13 MRSP isolates displayed MDR (Fig. 3). Indeed, all of the strains were simultaneously resistant to at least penicillin, tetracycline, sulfamethoxazole/trimethoprim, and clindamycin (Table 2).

DISCUSSION

In this study, a high prevalence of MDRSP and MRSP was found among clinical isolates of canine pyoderma and otitis externa in Korea. Among 74 *S. pseudintermedius* isolates, 45 (60.8%) and 13 (17.6%) were MDRSP and MRSP, respectively. In particular, all 13 of the *mecA*-positive MRSP isolates displayed simultaneous resistance to multiple antibiotics other than β-lactams.

Methicillin resistance in staphylococcal species is induced by the production of the low-affinity penicillin-binding protein PBP2a encoded by *mecA* on the genetic element called staphylococcal chromosome cassette *mec* (SCC*mec*) [12, 14]. Among the different types of SCC*mec*s, Type II is known to be the most likely source of

Table 2. Antimicrobial susceptibility of 13 methicillin-resistant *S. pseudintermedius* isolates.

Antimicrobial agent	Number of *S. pseudintermedius* isolates (%)		
	Resistant	Intermediate	Susceptible
Penicillin	13 (100)	0 (0)	0 (0)
Tetracycline	13 (100)	0 (0)	0 (0)
Sulfamethoxazole/Trimethoprim	13 (100)	0 (0)	0 (0)
Erythromycin	11 (84.6)	0 (0)	2 (15.4)
Clindamycin	13 (100)	0 (0)	0 (0)
Gentamycin	12 (92.3)	0 (0)	1 (7.7)
Chloramphenicol	11 (84.6)	0 (0)	2 (15.4)
Amoxicillin/Clavulanic Acid	6 (46.2)	0 (0)	7 (53.8)

transmission between animals and humans by direct contact [15]. However, a recent study demonstrated that fluoroquinolone- and methicillin-resistant *S. pseudintermedius* strains harbor two novel types of SCC*mec* elements, namely SCC*mec* II–III and SCC*mec* VII, which belong to class A/allotype 3 and class A/allotype 5, respectively [3]. Furthermore, Sasaki *et al*. [20] have reported that high-level oxacillin-resistant MRSP isolates carry SCC*mec* Type III. Therefore, it would be of interest to determine the type of SCC*mec* elements among the 13 MRSP isolates in the present study.

Previously, MDR has been frequently associated with methicillin-resistant *S. aureus* (MRSA) or methicillin-resistant *S. intermedius* (MRSI) isolates in humans or animals and has also been reported in *S. intermedius* without methicillin resistance. However, a recent epidemiological study suggested the emergence and spread of MRSI with MDR [10]. Although limited information is available on MDRSP carrying methicillin resistance, the results of the present study imply a high frequency in canine patients with pyoderma and otitis externa in Korea. This prevalence may be due to the lack of strict regulation of antibiotics in pet clinics and/or administration of antibiotics by some dog breeders and pet owners.

Previously, a preferable correlation was shown between the detection of *mecA* by PCR and oxacillin resistance by disk diffusion test in *S. aureus* [4, 12]. However, some staphylococcal isolates have been reported to be *mecA*-positive, oxacillin-susceptible [13, 18] or *mecA*-negative, oxacillin-resistant [18]. In the present study, three *S. pseudintermedius* isolates (6.1%) displayed a *mecA*-negative, intermediate oxacillin-resistant phenotype, whereas nine isolates (48.0%) showed a *mecA*-positive, oxacillin-susceptible phenotype. Although the exact mechanism behind this observation may require further investigation, it has been proposed that in *S. aureus* hyperproduction of β-lactamase or PBPs with altered activity are involved [24]. Alternatively, variable expression of *mecA* might be possible [6]. Since PCR detection of *mecA* has been considered the gold standard for detection of methicillin-resistant staphylococcal species, these results strongly suggest that oxacillin resistance by disk diffusion should be recommended for the determination of methicillin resistance.

Recently, MRSP infections have been reported in dogs [7, 20, 21]. In Korea, the high frequency of antibiotic-resistant bacteria has been of great concern in veterinary practice [18, 25] as well as in human medicine [11]. The prevalence of MRSP and MDRSP in pet dogs can be a potential hazard to public health; zoonotic transmission of such strains between pet dogs and their owners is possible, and the horizontal transfer of antibiotic-resistant genes such as SCC*mec* is also likely to occur. Currently, antibiotic usage by humans has been strictly controlled by Korean national policy, resulting in a reduction in inappropriate prescriptions for antibiotics against infections [19]. Continuous monitoring would be required to elucidate whether such a measure results in a decrease in bacterial resistance to certain antibiotics. In conclusion, our results emphasize the importance of judicious selection of antibiotics for small animal patients such as pet dogs by veterinary staffs, especially when long-term treatment is required.

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