High Prevalence of Fluoroquinolone- and Methicillin-Resistant *Staphylococcus pseudintermedius* Isolates from Canine Pyoderma and Otitis Externa in Veterinary Teaching Hospital

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Received: October 30, 2009 / Revised: December 16, 2009 / Accepted: December 18, 2009

Recently, a total of 74 *Staphylococcus pseudintermedius* isolates were collected from clinical cases of canine pyoderma and otitis externa in Korea. In this study, we examined *in vitro* fluoroquinolone resistance among those isolates using a standard disc diffusion technique. The results demonstrated that, except for one isolate, approximately 18.9% to 27.0% of the isolates possessed bacterial resistance to both veterinary- and human-licensed fluoroquinolones including moxifloxacin (18.9% resistance), levofloxacin (20.3% resistance), ofloxacin (24.3% resistance), ciprofloxacin (25.7% resistance), and enrofloxacin (27.0% resistance). Most surprisingly, 14 out of 74 (18.9%) isolates were resistant to all the five fluoroquinolones evaluated. Moreover, a PCR detection of the methicillin resistance gene (*mecA*) among the 74 isolates revealed that 13 out of 25 (52.0%) *mecA*-positive isolates, but only 7 out of 49 (14.3%) *mecA*-negative isolates, were resistant to one or more fluoroquinolones. Taken together, our results imply that bacterial resistance to both veterinary- and human-use fluoroquinolones becomes prevalent among the *S. pseudintermedius* isolates from canine pyoderma and otitis externa in Korea, as well as that the high prevalence of the *mecA*-positive *S. pseudintermedius* isolates carrying multiple fluoroquinolones resistance could be a potential public health problem.

Keywords: *Staphylococcus pseudintermedius*, fluoroquinolone, antibiotic resistance, dogs, *mecA* gene, prevalence

*Staphylococcus pseudintermedius* is an opportunistic bacterial pathogen that causes various dermatological diseases in dogs, and is known to be responsible for approximately 80% of canine pyoderma cases worldwide [21]. The microorganism has been differentiated from *S. intermedius* by a recent genotypic reclassification [5]. Interestingly, the first case of human infection by this microorganism [26] as well as the emergence and prevalence of methicillin-resistant populations [23] have been reported, implying potential cross-transmission between pet animals and humans.

Fluoroquinolone is a class of antimicrobials that are effective against a broad range of Gram-positive and -negative bacteria and, therefore, has been commonly used in humans and animals as antimicrobial therapeutics. The efficacy of fluoroquinolone in veterinary fields against the *S. intermedius* infection has been approved. For example, fluoroquinolones are recommended as a primary antibiotic regimen for canine pyoderma caused by *S. intermedius* [9]. However, gradual increases of fluoroquinolone resistance have been reported in *S. intermedius* isolates from canine pyoderma, otitis externa [10, 11, 15], and urinary tract infections [4], which might be due to extensive administration, unnecessary overdose, and/or prolonged misuse of fluoroquinolones [10, 22]. Several fluoroquinolones including enrofloxacin, difloxacin, orbifloxacin, and marbofloxacin are licensed for veterinary use [9]. Although ciprofloxacin and ofloxacin have been licensed for veterinary use in Korea, it is known that their use has been restricted to humans in many countries including the United States. Moreover, human-use fluoroquinolones such as levofloxacin and moxifloxacin are not recommended for animals. However, their chemical and structural similarities with veterinary-use fluoroquinolones may induce simultaneous resistance to both veterinary- and human-use fluoroquinolones.

In this study, we examined bacterial resistance of the 74 *S. pseudintermedius* isolates from canine pyoderma or otitis externa in Korea against the five fluoroquinolones;
one veterinary-licensed fluoroquinolone (enrofloxacin) and the other four human-licensed ones (ciprofloxacin, ofloxacin, levofloxacin, and moxifloxacin). In addition, fluoroquinolone resistance among the methicillin-resistant \textit{S. pseudintermedius} isolates was investigated by the detection of the methicillin resistance gene (\textit{mecA}) by polymerase chain reaction (PCR).

**Materials and Methods**

**Isolation and Identification of \textit{S. pseudintermedius}**

A total of 74 \textit{S. pseudintermedius} isolates were obtained from dog patients with pyoderma or otitis externa, referred to our veterinary teaching hospital in Konkuk University from local animal clinics during the period of 2006–2008. Signalments of canine patients are summarized in Table 1. Unfortunately, details on medical history such as pretreatment of antibiotics were not available. However, six of them have not been exposed to fluoroquinolones. The other cases were referred from local animal hospitals and thus it is believed that they might be exposed to antibiotics.

Cotton swab specimens were collected from the canine skin or ear lesions and inoculated onto 5% (v/v) sheep blood agar plates. They were then incubated at 37°C for 24 to 48 h. Primary identification of staphylococci was made on the basis of colony morphology, Gram-staining, and conventional catalase test. The staphyloccocal isolates were further tested for coagulase synthesis, lack of colony pigmentation, acetoin production, presence of β-galactosidase, and the PCR using the \textit{S. intermedius}-specific primers as previously described [29]. Confirmative identification of \textit{S. pseudintermedius} was done by the PCR–RFLP (restriction fragment length polymorphism) method recently developed by Bannoehr et al. [2].

**Determination of Fluoroquinolone Resistance**

All 74 \textit{S. pseudintermedius} isolates were examined for their resistance to the five major fluoroquinolones as listed below, using a standard disc diffusion method, which followed the Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. The fluoroquinolones and their disc contents were as follows: ciprofloxacin (5 µg; Becton, Dickinson and Company, U.S.A.), ofloxacin (5 µg; Becton, Dickinson and Company, U.S.A.), enrofloxacin (5 µg; Bayer HealthCare AG Germany), levofloxacin (5 µg; Becton, Dickinson and Company, U.S.A.), and moxifloxacin (5 µg; Becton, Dickinson and Company, U.S.A.). Enrofloxacin is one of the widely used quinolones that have been generally used for human diseases.

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**Detection of \textit{mecA} Gene by PCR**

The presence or absence of the \textit{mecA} gene among the \textit{S. pseudintermedius} isolates was determined by the PCR using the \textit{mecA}-specific primers as previously described by Zubeir et al. [29].

**Results**

The results demonstrated that among the 74 \textit{S. pseudintermedius} isolates, bacterial resistance was observed in 20, 19, 18,

### Table 1. Signalments of canine patients in this study.

<table>
<thead>
<tr>
<th>Skin disease</th>
<th>Number (N=56)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>31</td>
<td>55.4</td>
</tr>
<tr>
<td>Superficial pyoderma</td>
<td>16</td>
<td>28.6</td>
</tr>
<tr>
<td>Food allergy</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Pododermatitis</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Alopecia X</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Bacterial folliculitis</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Contact dermatitis</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>Eosinophilic fruncioles</td>
<td>1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*NA: Not available.

Briefly, \textit{S. pseudintermedius} strains were cultured in Mueller–Hinton (MH) broth for 6–8 h. Bacterial cultures were adjusted to turbidity of 0.5 McFarland standard and inoculated on the MH agar plates using a cotton swab. The plates were then incubated for 24 h at 37°C. \textit{S. aureus} ATCC 25923 and \textit{E. coli} ATCC 25922 strains were used as a quality control for disk diffusion assay. According to the interpretative standards of Clinical Laboratory Standards Institute (CLSI) [18, 19], bacterial resistance was categorized into “Susceptible,” “Intermediate resistant,” and “Resistant.” The percentage of the resistant populations against individual fluoroquinolones was calculated and compared with each other. In this study, multiple resistance was defined as bacterial resistance to three or more antimicrobial agents belonging to different subclasses.

**Detection of the \textit{mecA} Gene by PCR**

The presence or absence of the \textit{mecA} gene among the \textit{S. pseudintermedius} isolates was determined by the PCR using the \textit{mecA}-specific primers as previously described by Zubeir et al. [29].

**Table 2. Fluoroquinolone resistance in the 74 \textit{S. pseudintermedius} isolates from canine pyoderma and otitis externa in Korea.**

<table>
<thead>
<tr>
<th>Pattern of resistance</th>
<th>CIP</th>
<th>OFX</th>
<th>ENR</th>
<th>LVX</th>
<th>MXF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>49</td>
<td>51</td>
<td>31</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>Intermediate</td>
<td>6</td>
<td>5</td>
<td>23</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Resistant</td>
<td>19</td>
<td>18</td>
<td>20</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

*See Materials and Methods.

*Abbreviations: CIP, ciprofloxacin; OFX, ofloxacin; ENR, enrofloxacin; LVX, levofloxacin; MXF, moxifloxacin.
The pattern of fluoroquinolone resistance among the high prevalence (13/25 isolates; 52.0%) of fluoroquinolone-resistant isolates showed resistance to two or more fluoroquinolones licensed for both veterinary (enrofloxacin, ciprofloxacin, and ofloxacin) and human use (ciprofloxacin, ofloxacin, levofloxacin, and moxifloxacin), including 14 isolates resistant to all the five fluoroquinolones. To our knowledge, this is the first report on the prevalence of fluoroquinolone resistance as well as the presence of the mecA gene among S. pseudintermedius isolates from canine pyoderma and otitis externa.

In this study, fluoroquinolone resistance among the S. pseudintermedius isolates from canine pyoderma or otitis externa was greatly higher (18.9–27.0%) than those previously reported in other countries (less than 10%) such as Canada (6–8%) [8], France (2%) [6], the United Kingdom (0.7–1.2%) [15], Australia (0%) [7], Italy (1.5%) [10], Czech (0%) [17], Denmark (1.2%) [20], and Japan (0%) [24]. The observed high prevalence of fluoroquinolone resistance could be explained by the lack of strict regulation over fluoroquinolones in veterinary use and/or self-medication of fluoroquinolones by some dog breeders and pet owners in Korea. Supporting this notion, it has been recently reported that fluoroquinolone resistance is increased owing to antibiotics abuse in the farm animal industry [14] as well as in human medicine [13] in Korea.

On the basis of their antimicrobial activities and chemical structures, fluoroquinolones can be categorized into the second, third, and fourth generations. Certain mutations in their target proteins such as DNA gyrase or reduced uptake of those antibiotics are known to contribute to the development of bacterial resistance [22]. However, some fluoroquinolones such as moxifloxacin and gatifloxacin appear to slow the development of bacterial resistance, especially in Gram-positive bacteria species, because the two mutations are generally required for developing bacterial resistance [1,22]. In this study, it was found that the highest resistance was observed with the veterinary-licensed enrofloxacin (27.0%), whereas the lowest was the human-licensed moxifloxacin (18.9%). Although several veterinary-licensed fluoroquinolones were introduced and recommended to use only in animals, their similarity in the chemical structures to human-licensed fluoroquinolones might induce cross-resistance against both veterinary- and human-use fluoroquinolones. As expected, 19 out of 20 (95.0%) fluoroquinolone-resistant isolates possessed resistance to two or more fluoroquinolones for both veterinary and human uses. Most surprisingly, 14 (73.7%) out of those 19 multi resistant S. pseudintermedius isolates were resistant to all the five fluoroquinolones tested, despite rare use of third or fourth generation fluoroquinolones such as moxifloxacin. These results imply that cross-resistances not only between veterinary- and human-use fluoroquinolones but also between rarely used third or fourth-generation fluoroquinolones can be observed. Therefore, measures such as appropriate use and restriction of fluoroquinolones are necessary to slow down the development of bacterial resistance [22].
fluoroquinolones are common among *S. pseudintermedii* isolates. Therefore, more specific care should be taken, and the antibiotic susceptibility test should be preceded before the prescription of fluoroquinolones.

Recently, a public health concern for multiple resistant *Staphylococcus* spp. against both methicillin and fluoroquinolones has been raised because of the restriction of antibiotic choice for staphylococcal infection [28]. Close association of methicillin-resistant *S. pseudintermedii* with high-level fluoroquinolone resistance has been reported [16], which is comparable to our results. Although the mechanisms behind the emergence of both fluoroquinolone- and methicillin-resistant *Staphylococcus* spp. are not clear, it has been proposed that inappropriate use of fluoroquinolones may potentiate bacterial adhesion mediated by the fibronectin-binding protein, which subsequently facilitates colonization may potentiate bacterial adhesion mediated by the fibronectin-binding protein, which subsequently facilitates colonization and increased adhesion of quinolone-resistant *Staphylococcus aureus* by subinhibitory levels of ciprofloxacin. *Antimicrob. Agents Chemother.* 44: 1428–1437.


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**Acknowledgments**

We are very grateful to Dr. Yong-Seung Park from Bayer Animal Health Care (Seoul, Korea) for his kind supply of enrofloxacin susceptibility discs. This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea Government (MEST) (R11–2002-103).


