

Antimicrobial resistance and novel mutations detected in the *gyrA* and *parC* genes from *Pseudomonas aeruginosa* strains isolated from companion dogs

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Abstract

Background Fluoroquinolone agents, such as enrofloxacin and marbofloxacin, are commonly used in pseudomonal infection in veterinary medicine. However, the resistance rate to fluoroquinolone is rapidly increasing according to multiple studies in various countries. The point mutation in quinolone resistance determining region (QRDR) is closely related to increased fluoroquinolone resistance of *Pseudomonas aeruginosa*. The aim of this study was to investigate current antimicrobial susceptibility and fluoroquinolone resistance in *Pseudomonas aeruginosa* strains isolated from dogs. The presence of the point mutations in the QRDR was confirmed by *gyrA* and *parC* polymerase chain reaction and nucleotide sequencing analysis. Results A total of 84 nonduplicated *P. aeruginosa* isolates were obtained from 228 healthy dogs (healthy group) and 260 dogs with clinical signs (infected group). From these, 38 isolates from the healthy group were detected in several samples, whereas 46 isolates from the infected group were mostly obtained from dogs' ears with otitis externa (41/260, 15.8%). All isolates were resistant to nalidixic acid, while some were also resistant to enrofloxacin (23/84, 27.4%), marbofloxacin (17/84, 20.2%), levofloxacin (12/84, 14.3%), or ciprofloxacin (11/84, 13.1%). Enrofloxacin resistance was significantly higher in strains from the infected group than that of the healthy group ($p < 0.05$). Among the 23 fluoroquinolone-resistant isolates, 8 and 4 different mutations were detected in the *gyrA* and *parC* genes, respectively. Mutations in *gyrA* were significantly common in the infected group ($p < 0.05$). Hotspots for the *gyrA* and *parC* mutations were Thr83 (34.8%, 8/23) and Pro116 (91.3%, 21/23), respectively. Double and triple mutations were also found in 5 of the isolates. Conclusion Novel mutations in the *gyrA* and *parC* genes were first found in *P. aeruginosa* isolates from companion dogs in South Korea. These findings suggest that it is important to know the prudent use of fluoroquinolone antibiotics in canine pseudomonas infection treatment.

Background

Pseudomonas aeruginosa is a gram-negative opportunistic bacterium that usually infects the skin and the urinary and respiratory tracts. In veterinary medicine, fluoroquinolones, such as enrofloxacin and marbofloxacin, are commonly prescribed when a pseudomonas infection is suspected; most commonly, in otitis externa/media or urinary tract infections. However, caution should be exerted in the use of antimicrobials to treat pseudomonas infections because of the increasing emergence of antimicrobial-resistant bacteria. The inappropriate use of antimicrobials, increasing prevalence of chronic illness, and lack of environmental barriers between animals and humans has not only contributed to the development of antimicrobial-resistant bacteria, but also impose a variety of selective pressures that bacteria must face and contend with. (1-3)

Quinolone is a broad-spectrum bactericide that inhibits bacterial DNA gyrase and type IV topoisomerase. In human medicine, the trend of *P. aeruginosa* resistance against quinolone is closely evaluated and monitored. According to the ECDC publishes 2014 surveillance data on antimicrobial resistance and antimicrobial consumption in Europe report, the incidence of fluoroquinolone-resistant *P. aeruginosa* is decreasing in many European countries, the United States, and Canada owing to the development of

guidelines for appropriate antimicrobial use by the European Center for Disease Prevention and Control. (4) The fluoroquinolone resistance profile of *P. aeruginosa* strains present in companion dogs has also been evaluated in the United States, France, Brazil, Croatia, and Japan. (5-9) Resistance to fluoroquinolones differs from one country to another; *e.g.*, resistance to marbofloxacin is higher in the United States than in Croatia (27% vs. 8.9%). In a previous study conducted in Croatia, resistance to marbofloxacin was lower there than in other countries because the use of marbofloxacin is only licensed for otitis externa. (9) Nevertheless, resistance to marbofloxacin in Croatia has increased since 2002, as marbofloxacin was commercially approved in Croatia soon afterwards (4.4% vs. 8.9%). (10, 9) The fluoroquinolone resistance profile of *P. aeruginosa* in companion dogs has not been evaluated in South Korea, although enrofloxacin and marbofloxacin are commonly prescribed in veterinary medicine.

P. aeruginosa develops resistance to quinolone through the following mechanisms: 1) quinolone resistance-determining region (QRDR); 2) plasmid-mediated quinolone resistance (PMQR); and 3) efflux pumps. QRDR is a chromosomal point mutation of either DNA gyrase (*gyrA* and *gyrB*) or type IV topoisomerase (*parC* and *parE*), which is the main mechanism of resistance to quinolone in *P. aeruginosa*. (6, 11) A previous study performed in the United States reported that half of the 102 (51.0%) nalidixic acid-resistant *P. aeruginosa* strains isolated from dogs had QRDR mutations. (6) QRDR mutations were also identified in fluoroquinolone-resistant *P. aeruginosa* from dogs in Brazil and Japan. (7, 8) However, the presence of QRDR mutations in dogs has not been reported in South Korea. This study was performed to investigate the antimicrobial resistance and mutations in *gyrA* and *parC* in fluoroquinolone-resistant isolates of *P. aeruginosa* from companion dogs in South Korea.

Methods

Sample collection

To investigate the antimicrobial susceptibility and fluoroquinolone resistance mechanisms of *P. aeruginosa* strains isolated from companion dogs with or without present clinical signs, sampling was performed on a total of 50 veterinary hospitals from five Korean provinces between 2017 and 2018. Samples were collected from the ears, nasal cavity, eyes, genitalia, rectum, and pus of dogs that were not ill and had not taken any antibiotic within the last six months, using sterile transport swabs (COPAN, Brescia, Italy), and of dogs with clinical signs of bacterial infection. Isolates were first classified according to their origin as *either belonging to the healthy group or to the infected group*. All samples collected from companion animals were carried out under the owner's approval. Every individual was provided with information regarding the purpose and method of sampling. The need for ethics approval was deemed unnecessary according to the national regulations.

Isolation and identification

Swab samples were cultured on trypticase soy broth (TSB, Becton, Dickinson and Company, Sparks, MD, USA) for 18h at 37°C. Then, they were streaked on MacConkey agar (Becton, Dickinson and Company) and incubated for 18 h at 37°C. Those isolates that were identified as Pseudomonas aeruginosa by Gram

staining (Gram stain set, Remel, Dartford, UK), oxidase tests (Becton, Dickinson and Company), and the microbial identification system (VITEK[®] MS, bioMérieux, Marcy-l'Étoile, France) were selected for further analysis.

Determination of antimicrobial susceptibility and minimum inhibitory concentrations

Antimicrobial susceptibility analyses were performed by the disk diffusion test, according to the Clinical Laboratory of Standard Institute guidelines. (12) Eleven different antimicrobial disks were used (Oxoid Limited, Basingstoke, UK) for testing: piperacillin, piperacillin-tazobactam, cefepime, ceftazidime, ciprofloxacin, gentamicin, amikacin, tobramycin, meropenem, imipenem, and colistin.

The minimum inhibitory concentrations (MICs) for fluoroquinolone antimicrobials were determined as indicated by the CLSI broth microdilution method. The quinolones and fluoroquinolones used were nalidixic acid (Sigma-Aldrich, Germany), ciprofloxacin (Sigma-Aldrich), levofloxacin (Sigma-Aldrich), enrofloxacin (Bayer Vital GmbH, Leverkusen, Germany), and marbofloxacin (Vetoquinol, Lure, France). *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains. The breakpoints for ciprofloxacin and levofloxacin were determined following the CLSI guidelines, whereas breakpoint for nalidixic acid was determined as described by Rubin et al., 2008, and enrofloxacin and marbofloxacin, as described by Pintarić et al., 2017.

PCR amplification and sequencing analysis

Genomic DNA was extracted from all *P. aeruginosa* isolates using a QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. PCR amplification of the *gyrA* and *parC* genes was performed as previously described (Rubin *et al.*, 2008). Purified PCR products were sequenced using the automated 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and resulting sequences were compared against the complete genome of *Pseudomonas aeruginosa* ATCC 27853 (GenBank accession number, CP015117), using both the BLAST network service and the ClustalW multiple sequence alignment program (www.genome.jp/tools-bin/clustalw).

Statistical analysis

The obtained data are presented as the number or percentage of prevalence from the values. A *t*-test and a Pearson's Chi-square test were used to compare the antimicrobial susceptibility profiles between the healthy group and the infected group. All statistical analyses were performed by using the SPSS 20.0 software (IBM SPSS, Inc., Chicago, IL, USA). Results were considered statistically significant for *p* values less than 0.05.

Nucleotide sequence accession numbers

Newly described sequences for the *gyrA* and *parC* genes have been assigned the following GenBank accession numbers: MN068218 to MN068220 (*parC* mutations) and MN068221 (*gyrA* mutation).

Results

Prevalence of P. aeruginosa strains isolated from companion dogs

A total of 84 nonduplicated *P. aeruginosa* strains were isolated from 448 companion dogs either with or without clinical signs from 2017 to 2018. From these, 38 isolates (16.7%) from 228 healthy dogs (healthy group) were detected in the ears (6.1%; 14/228), the eyes (4.4%; 10/228), the nasal cavity, and the rectum (3.1%; 7/228). Whereas, 46 isolates (17.7%) from 260 dogs with clinical signs (infected group) were mostly present in the ears (15.8%, 41/260), and only a few were found in the genitalia (1.5%; 4/260), and in pus (0.4%; 1/260) (Table 1). Thereafter, *P. aeruginosa* was mostly isolated from ear samples (55/448, 11.3%) of companion dogs which are independent of their health status.

Antimicrobial resistance profile

Isolates were tested on 11 different antibiotics to determine whether they were resistant to them (Table 2). In the healthy group, the resistance to ciprofloxacin was the highest ($n=4$), and the resistance to ciprofloxacin-gentamicin-tobramycin, gentamicin, and tobramycin was one organism, respectively. In the infected group, the resistance to ciprofloxacin was the highest ($n=7$), followed by piperacillin ($n=3$), ciprofloxacin-gentamicin-tobramycin ($n=2$), gentamicin ($n=2$), tobramycin ($n=2$) and amikacin ($n=1$). No isolate was resistant to cefepime, ceftazidime, colistin, imipenem, meropenem, or piperacillin-tazobactam. Additionally, resistance to piperacillin and amikacin was observed only in isolates from the infected group. While resistance to ciprofloxacin was apparently higher in the infected group, there was no significant difference in the resistance frequency to ciprofloxacin between the healthy and the infected groups (p -value 0.747).

The MICs for 4 fluoroquinolone antibiotics, including nalidixic acid, were evaluated (Table 3). All *P. aeruginosa* isolates from both two groups were resistant to nalidixic acid (MICs ranges: 64 to ≥ 256 $\mu\text{g}/\text{mL}$ in the healthy group and 32 to ≥ 256 $\mu\text{g}/\text{mL}$ in the infected group) (data not shown). While the highest percentage of resistance among the 4 fluoroquinolone agents was seen for enrofloxacin (breakpoint, ≥ 4 $\mu\text{g}/\text{mL}$), with 15.8% (6/38) resistant isolates from the healthy group and 37.0% (17/46) from the infected group. The percentage of resistance to marbofloxacin (≥ 4 $\mu\text{g}/\text{mL}$) was 13.2% (5/38) for isolates from the healthy group and 26.1% (12/46) for those from the infected group, and resistance (≥ 8 $\mu\text{g}/\text{mL}$) to levofloxacin was 13.2% (5/38) for the healthy group isolates and 15.2% (7/46) for those from the infected group. Moreover, the resistance (≥ 4 $\mu\text{g}/\text{mL}$) to ciprofloxacin was of 10.5% (4/38) in isolates from the healthy group and 15.2% (7/46) in isolates from the infected group (Table 3). Collectively, isolates from the infected group were significantly more resistant to enrofloxacin and less susceptible to marbofloxacin than those from the healthy group ($p < 0.05$).

Amino acid variations in gyrA and parC genes

Given that isolates resistant to enrofloxacin among fluoroquinolone antibiotics were the most abundant, the sequencing analysis of the *gyrA* and the *parC* genes from the 23 enrofloxacin-resistant *P. aeruginosa*

isolates was performed (Table 4). QRDR mutations were identified in 6 (15.8%) out of 38 isolates from the healthy group and in 17 (37%) out of 46 isolates from the infected group; all these 23 mutations belonged to the 23 enrofloxacin-resistant isolates of *P. aeruginosa*. Besides, 11 (47.8%) out of these 23 isolates were resistant to ciprofloxacin. From the amino acid substitutions found in the 23 fluoroquinolone-resistant isolates, 8 were present in the *gyrA* gene and 4 in the *parC* gene. In relation to *gyrA*, the mutation Thr83Ile was found in 2 isolates from the healthy group and in 4 isolates from the infected group; besides, other mutations were found in isolates from the infected group: Asp87Gly in 2 isolates, Thr83Ile-Asp87Gly in 1 isolate, Leu55Gln-Asp82Asn-Thr83Ala in 1 isolate, and Asp87Asn in 1 isolate. Noteworthy, the novel triple-nucleotide mutation found in *gyrA*, leading to codon changes Leu55→Gln, Asp82→Asn, and Thr83→Ala, corresponds to a *P. aeruginosa* isolate from the infected group with high MIC values for the four fluoroquinolone agents tested. Moreover, the strain with two nucleotide mutations, *i.e.*, Thr83Ile and Asp87Gly, showed identical MIC values for these four antibiotics. Mutations in *gyrA* were significantly more common in strains from the infected group than from the healthy group ($p < 0.05$).

Regarding the *parC* gene, mutations were observed in all 23 enrofloxacin-resistant *P. aeruginosa* isolates. A novel single nucleotide mutation at *parC* codon 116, Pro116→Arg, was found in 21 (91.3%) of the isolates, which included a double nucleotide alteration at codons 87 and 116, Ser87→Leu and Pro116→Arg, that was observed in 1 isolate from the healthy group and in 2 isolates from the infected group. In addition, a frameshift (fs) mutation due to a nucleotide deletion at *parC* codon 116, Pro116fs, CGG→CG-, was detected in 2 isolates from the infected group. Therefore, mutation hotspots found for *gyrA* and *parC* were Thr83Ile (n=7) and Pro116Arg (n=21), respectively. There was no significant difference between the number of point mutations in either the *gyrA* or the *parC* genes and the MIC values to fluoroquinolone antimicrobial agents among resistant isolates. In addition, QRDR mutations in the *gyrA* or the *parC* genes conferring an intermediate resistance to enrofloxacin (MIC: 1 to 2 µg/mL) were not found in *P. aeruginosa* isolates.

Discussion

Pseudomonas aeruginosa often causes otitis externa/media and cystitis in companion dogs. Despite the regular usage of fluoroquinolone agents such as enrofloxacin and marbofloxacin in the treatment of infected dogs, neither the antimicrobial susceptibility nor the quinolone-resistance profile of *P. aeruginosa* strains has ever been studied in South Korea before. This study aimed to reveal the antimicrobial susceptibility and quinolone resistance of *P. aeruginosa* strains isolated from both healthy and clinically ill companion dogs. *P. aeruginosa* was recovered from several samples in dogs of the healthy group; nevertheless, one-third of these isolates were obtained from the ears. In addition, *P. aeruginosa* was mainly isolated from the ears of dogs from the infected group. These results are consistent with *P. aeruginosa* as a common agent causing otitis and cystitis. The fact that one-third of the isolates from the healthy group were obtained from the ears suggests a history of otitis externa/media in dogs that appear to be healthy.

P. aeruginosa isolates from this study were not resistant to a variety of antimicrobials, tested by disk diffusion assays, except for ciprofloxacin, gentamicin, tobramycin, and amikacin. The resistance to these latter antimicrobial agents may be due to the prevalence of use of fluoroquinolones and aminoglycosides for treating diseases in animal hospitals. It is important to note that a significant difference in the resistance frequencies to ciprofloxacin between healthy and infected group was not found. These results could be explained if the usage of ciprofloxacin, among quinolone antibiotics, is less common in animal hospitals for treating diseases; then, it would be possible to hypothesize that the companion dogs in this study had not been exposed to ciprofloxacin.

MIC values to fluoroquinolones were different among antibiotics. All isolates showed high resistance to enrofloxacin but low resistance to ciprofloxacin. These results are similar to *P. aeruginosa* resistance trends reported in other countries. The previous resistance reports indicate that 13.1% isolates from this study, 16% from the United States, 13% from Spain, 2.2% from Croatia, 4.8% from Brazil, and 20% from Japan are resistant to ciprofloxacin. (6-9) Moreover, reported percentage of resistance to enrofloxacin and marbofloxacin are 27.4% and 20.2% in this study, 31% and 27% in the United States, 21.7% and 9% in Spain, and 15.6 and 8.9% in Brazil, respectively, whereas a 31.5% resistance to enrofloxacin was reported in Japan. According to a previous study, *P. aeruginosa* isolates show their highest resistance to enrofloxacin, the most commonly used fluoroquinolone in veterinary medicine. (6) In Croatia, resistances to enrofloxacin and marbofloxacin significantly increased from 2.7% to 15.6% and from 4.4% to 8.9%, respectively, since the use of marbofloxacin, besides enrofloxacin, in veterinary medicine was approved in the country. (10, 9) In contrast, resistance to ciprofloxacin decreased from 3.8% to 2.2% because of a reduction in the usage of this antibiotic. Our results show that resistance to marbofloxacin was 20.2%, which is higher than that reported for strains from Spain and Croatia. (8, 9) This finding may be related to differences in the frequency of marbofloxacin prescription in each country. Since the usage of marbofloxacin for treating clinically ill dogs was approved in South Korea, follow-up studies should be undertaken to determine the evolution of fluoroquinolone-resistant *P. aeruginosa* strains.

Previous studies have also reported different susceptibility or resistance to enrofloxacin depending on the origin of studied isolates; ear isolates, for example, show a significantly higher resistance to enrofloxacin than skin isolates. (13) Other studies have reported increased resistance rates in ear isolates. (14, 6) In Croatia, resistance to enrofloxacin increased from 1.0% in 2011 to 8.9% in 2017, maybe because Mekić and collaborators only evaluated the resistance of isolates from dogs with otitis externa. (15, 9) Ear isolates from this study showed a high susceptibility to ciprofloxacin (82.5%), and a low susceptibility both to enrofloxacin (17.5%) and marbofloxacin (3.5%). This trend is similar to that reported in Croatia, where the use of marbofloxacin has only been licensed for treating otitis externa. As mentioned, in this study, ear isolates were significantly less susceptible to marbofloxacin than isolates from other body parts ($p < 0.05$). This finding suggests that caution should be taken in the usage of enrofloxacin or marbofloxacin in dogs with otitis in order to avoid an increase in resistance rates to these antibiotics.

In the present study, novel QRDR mutations in both the *gyrA* and the *parC* genes were detected in fluoroquinolone-resistant isolates of *P. aeruginosa*. Among QRDR genes (*gyrA*, *gyrB*, *parC*, and *parE*),

QRDR mutations are most frequently detected in *gyrA* and *parC*. (6-8) In this study, the same point mutation within *parC* was found in all enrofloxacin-resistant isolates, whereas point mutations in *gyrA* were almost exclusively detected in isolates from the infected group. These results suggest a high correlation between QRDR mutations and increased resistance to fluoroquinolones, because strains from the infected group were significantly more resistant to enrofloxacin than those from the healthy group. The most commonly reported point mutations or hotspots are Thr83 and Asp87 for *gyrA* and Ser87 for *parC* both in dogs and humans. (6, 8, 16) Consistently, the hotspot for *gyrA* was Thr83 (n=8, 38.1%) in this study. In contrast, a point mutation at Pro116 of *parC* (n=21, 80.8%) in this study was the most frequently observed mutation found in isolates from this study. This may be an alternative form of the gene, specific to canine *P. aeruginosa* isolates from South Korea rather than a QRDR mutation resulting in increased resistance to fluoroquinolone. Nevertheless, a high correlation between QRDR mutations and increased MICs to fluoroquinolones has been reported previously. (7) In this study, the MIC values for the four fluoroquinolone antibiotics studied were not significantly different for isolates with different *gyrA* mutations, while *parC* mutations were detected in all 23 enrofloxacin-resistant isolates. As a result, the novel Pro116 alteration described in *parC*, which has never been reported in nalidixic-acid resistant Gram-negative bacteria until now, is suspected to be correlated with increased enrofloxacin resistance.

Conclusions

The high resistance to enrofloxacin and the occurrence of QRDR mutations in *P. aeruginosa* isolates from clinically ill dogs may reflect a common use of enrofloxacin in animal hospitals. Overall, this study warns about an inappropriate use of fluoroquinolone agents in the treatment of pseudomonas infections in companion dogs.

Abbreviations

Ala: Alanine; AMK: Amikacin; Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; CIP: Ciprofloxacin; CLSI: Clinical and Laboratory Standards Institute; Cys: Cystine; ECDC: European centre for disease prevention and control; ENR: Enrofloxacin; Fs: Frameshift; GEN: Gentamicin; Gln: Glutamine; Gly: Glycine; Ile: Isoleucine; Leu: Leucine; LVX: Levofloxacin; MFX: Marbofloxacin; MIC: Minimum inhibitory concentration; NAL: Nalidixic acid; PCR: Polymerase chain reaction; PIP: Piperacillin; Pro: Proline; QRDR: Quinolone resistance determining region; Ser: Serine; Thr: Threonine; TOB: Tobramycin; Tyr: Tyrosine

Declarations

Ethics approval and consent to participate

All samples collected from companion animals were carried out under the owner's approval. Every individual was provided with information regarding the purpose and method of sampling. The need for ethics approval was deemed unnecessary according to the national regulations.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Author's contributions

YJ Park performed the antibiotics susceptibility and identification of QRDR, analyzed all relevant data and mainly wrote this manuscript. JY Oh and HM Park guided and reviewed designing this experiment and edited the manuscript. SW Park, S Sum, WK Song, and JC Chae contributed to the isolation, identification, and antimicrobial susceptibility test (disk diffusion test only) of bacterial strains. All authors read and approved the final manuscript.

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Tables

Table 1

Distribution of nonduplicated isolates of *Pseudomonas aeruginosa* between two dog groups

Specimens	No. (%) of isolates	
	Healthy group (n=228)	Infected group (n=260) ^a
Eye	10 (4.4)	0 (0.0)
Rectum	7 (3.1)	0 (0.0)
Genitalia	0 (0.0)	4 (1.5)
Ear	14 (6.1)	41 (15.8)
Nasal cavity	7 (3.1)	0 (0.0)
Pus	0 (0.0)	1 (0.4)
Total	38 (16.7)	46 (17.7)

^a Disease name of the infected group: genitalia, Pyometra; ear, Otitis externa; pus, Bronchitis.

Table 2

Antimicrobial resistance patterns of two groups

Antimicrobial resistance pattern ^a	No. (%) of resistant strains		
	Healthy group (n=38)	Infected group (n=46)	Total (n=84)
PIP	0 (0.0)	3 (6.5)	3 (3.6)
CIP	4 (10.5)	7 (15.2)	11 (13.1)
CIP-GEN-TOB	1 (2.6)	2 (4.3)	3 (3.6)
AMK	0 (0.0)	1 (2.2)	1 (1.2)
GEN	1 (2.6)	2 (4.3)	3 (3.6)
TOB	1 (2.6)	2 (4.3)	3 (3.6)

^a PIP, piperacillin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin.

Table 3

Fluoroquinolone resistance rate among two dog groups

Antimicrobial agents	MIC ($\mu\text{g/mL}$)		No. (%) of resistant by		<i>p</i> -value
	Range	Breakpoint	healthy group (<i>n</i> =38)	infected group (<i>n</i> =46)	
Ciprofloxacin	0.015-32	≥ 4	4 (10.5)	7 (15.2)	0.747
Levofloxacin	0.015-32	≥ 8	5 (13.2)	7 (15.2)	0.700
Enrofloxacin	0.03-16	≥ 4	6 (15.8)	17 (35.4)	0.031 ^a
Marbofloxacin	0.03-16	≥ 4	5 (13.2)	12 (26.1)	0.142

^a Statistically significant ($p < 0.05$).

Table 4

Comparison of quinolone resistance determining regions among the fluoroquinolone-resistant *Pseudomonas aeruginosa* isolates between the healthy- and clinical dog groups

Strain no.	Amino acid substitution ^a		MICs (µg/mL) ^b			
	<i>gyrA</i>	<i>parC</i>	CIP	LVX	ENR	MFX
Healthy group						
PAE18	-	Pro116Arg	4	16	≥16	≥16
PAE43	Thr83Ile	Pro116Arg	2	8	≥16	8
PAE51	-	Pro116Arg	16	16	≥16	≥16
PAE52	Thr83Ile	Ser87Leu, Pro116Arg	≥32	≥32	≥16	≥16
PAE71	-	Pro116Arg	≥32	≥32	≥16	≥16
PAE85	-	Pro116Arg	0.25	0.5	4	1
Infected group						
KVNON14	Asp87Gly	Pro116Arg	4	8	≥16	≥16
KVNON23	Thr83Ile	Pro116Arg	≥32	≥32	≥16	≥16
KVNON33	Thr83Ile, Asp87Gly	Ser87Leu,Pro116Arg	≥32	≥32	≥16	≥16
KVNON47	-	Pro116fs	0.5	2	4	2
KVNON66	Asp87Gly	Pro116Arg	4	8	≥16	≥16
KVNON127	Asp87Tyr	Pro116Arg	1	2	4	4
KVNON184	-	Pro116Ala	0.125	0.5	8	1
KVNON194	Leu55Gln, Asp82Asn, Thr83Ala	Ser87Leu, Pro116Ala	≥32	≥32	≥16	≥16
KVNON199	Thr83Ile	Pro116Arg	8	32	≥16	≥16
KVNON216	-	Pro116Arg	0.5	2	4	2
KVNON219	Thr83Ile	Pro116Arg	1	4	8	8
KVNON271	-	Pro116Arg	2	4	4	2
KVNON279	Thr83Ile	Pro116Arg	4	8	≥16	≥16
KVNON324	-	Pro116Arg	0.5	4	4	4
KVNON442	Asp87Asn	Pro116Arg	0.5	2	4	4
KVNON508	-	Pro116fs	0.5	2	4	2
KVNON509	-	Pro116Arg	2	2	4	4

^a Amino acid substitutions in *gyrA* and *parC*: Thr83Ile, ACC → ATC; Thr83Ala, ACC → GCC; Asp87Gly, GAC → GGC; Leu55Gln, CTG → CAG; Asp82Asn, GAC → AAC; Asp87Asn, GAC → AAC; Asp87Tyr, GAC → TAC; Pro116Arg, CCG → CGG; Pro116Ala, CCG → GCG; Ser87Leu, TGC → TTG; Pro116fs, CGG → CG-. Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Gly, glycine; Ile, Isoleucine; Leu, leucine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; fs, frameshift.

^b NAL, nalidixic acid; CIP, ciprofloxacin; LVX, levofloxacin; ENR, enrofloxacin; MFX, marbofloxacin.