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Prevalence of Antimicrobial Resistance in Fecal *Escherichia coli* Isolates from Stray Pet Dogs and Hospitalized Pet Dogs in Korea

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A total of 628 *Escherichia coli* isolates recovered from 877 intestinal samples of stray pet dogs ($n = 565$) and hospitalized pet dogs ($n = 312$) in Korea were analyzed for resistance to 15 antimicrobial agents. Most common resistance observed in *E. coli* isolated from both groups of dogs was to tetracycline (52.4–53.6%), streptomycin (35.8–41.7%), ampicillin (32.9–47.1%), nalidixic acid (21.6–37.4%), and trimethoprim/sulfamethoxazole (19.7–36.4%). Resistance to chloramphenicol, gentamicin, and ciprofloxacin was observed in 19.4% (17.1–24.3%), 18% (16.1–21.8%), and 16.1% (13.5–21.4%) of the isolates, respectively. No *E. coli* isolated from hospitalized dogs showed resistance to imipenem and cefepime, whereas three (0.7%) isolates from stray dogs were resistant to cefepime. Some of the isolates from both groups showed resistance to cefotaxime (2.4–3.9%) and amikacin (0.5–1.5%). In general, the frequency of resistance tended to be higher in isolates from hospitalized dogs than isolates from stray dogs against most antimicrobials tested. Around 39% (162/422) and 27% (55/206) of *E. coli* isolates from stray dogs and hospitalized dogs were susceptible to all antimicrobials tested, respectively. Multiresistance ($\geq$3 subclasses of antimicrobials) was observed in 32% and 48% of *E. coli* isolates from stray dogs and hospitalized dogs, respectively. More attention should be paid to the use of antimicrobials and the occurrence of antimicrobial resistance in companion animals.

Introduction

Antimicrobial resistance in companion animals is an emerging problem.[19] Because antimicrobial use in companion animals is mainly focused on therapeutic use in individual animals,[21] patterns of antimicrobial use in companion animal medicine have more in common with antimicrobial use in human medicine than with food animal medicine.[6] Transmission of resistant bacteria or mobile resistance determinants between companion animals and humans has also been reported.[7,20] To understand the possible risks to humans, information is needed on antimicrobial resistance situation of the bacterial flora of companion animals. However, scientific data on the extent and importance of antimicrobial resistance in companion animal bacteria barely exist, in part because of limited surveillance.[19]

Antimicrobial-resistant bacteria in companion dogs may transfer their resistance genes to human commensal or pathogenic bacteria since dogs share the same environment as humans and contact closely with humans.[17] Very few investigations on antimicrobial resistance among bacterial isolates from dogs have been published in Korea, and most of them have focused on *Staphylococcus aureus*[13,16] or *Staphylococcus pseudintermedius*.[11] To our knowledge, however, there has been no previous study on antimicrobial resistance in fecal indicator bacteria from healthy or sick dogs in Korea. *E. coli* is commonly found in the intestinal flora of animals and humans, and known to be a very good indicator for selection pressure by antimicrobial use and for resistance problems to be expected in pathogens.[5] There is one study that has reported resistance among *E. coli* isolates from dogs admitted to a veterinary teaching hospital in Korea,[22] but those isolates were all from clinical samples such as skin, urine, ear canal, and conjunctiva, not from samples of intestine or feces. The objective of this study was, therefore, to assess the prevalence of antimicrobial resistances in fecal *E. coli* isolates recovered from intestinal samples of companion dogs that have been submitted by stray dog shelters and small animal clinics throughout the country.

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Materials and Methods

Sample collection

A total of 877 intestinal samples of pet dogs (565 stray dogs and 312 hospitalized dogs) were tested in this study. All the intestinal samples were obtained from dead dogs, which had been collected from stray animal shelters and veterinary clinics throughout the country during 2006–2007: each stray animal shelter and each two veterinary clinics located in 16 provinces and metropolitan cities in Korea. Stray dogs (401 euthanized and 164 died of natural causes) that died in shelters and individually owned pet dogs that died during hospitalization were refrigerated immediately after death and delivered as soon as possible to the diagnostic laboratory of National Veterinary Research and Quarantine Service, Ministry for Food, Agriculture, Forestry, and Fisheries of Korea. All the dogs included in this study were companion dogs: of 34 breeds of dogs, 20 most frequent breeds were common in both groups; the 6 most common breeds in both stray dog and hospitalized dog groups were Shih-tzu (25.1% and 20.8%), Maltese (12.2% and 19.9%), Yorkshire terrier (8.6% and 11.9%), Cocker spaniel (7.4% and 6.1%), Schnauzer (6.5% and 5.6%), and Poodle (6.3% and 5.1%). Information on antimicrobials used in hospitalized dogs was not available.

Isolation and identification of E. coli

Swabs of intestine were directly plated on Chromogenic *E. coli*/coliform agar (Oxoid, Basingstoke, Hants, England) and incubated overnight at 37°C. Colonies showing typical characteristics for *E. coli* on the agar (purple) were streaked onto eosin methylene blue (EMB) agar (Becton Dickinson, Sparks, MD) and incubated at 37°C for 18 to 20 hr. The EMB agar plates were examined and selected for metallic sheen colonies, which were streaked again on MacConkey agar (Becton Dickinson). After overnight incubation at 37°C, at least two typical pink colonies were selected and tested further by biochemical methods such as indole, methyl-red, Voges-Proskauer, and citrate test, and by the Vitek system (BioMerieux, Hazelwood, MO) for confirmation.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the agar disk diffusion methods[1] with 15 antimicrobial agents selected: ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, cefotaxime, cefepime, streptomycin, gentamicin, amikacin, ciprofloxacin, nalidixic acid, trimethoprim/sulfamethoxazole, chloramphenicol, tetracycline, and imipenem. Cartridges of antimicrobial-containing discs were obtained from Becton Dickinson (Sensi-Disk). Except for aminoglycoside, one antimicrobial was selected from each subclass of antimicrobials, which are either important in humans or commonly used in animal hospitals in Korea. Inhibition zone diameters were interpreted in accordance with the guidelines of the Clinical Laboratory Standards Institute.[2] Intermediate resistance was not regarded as resistance. *E. coli* ATCC 25922 and *E. coli* ATCC 35218 (for beta-lactam/beta-lactamase inhibitor) strains were included with each batch of specimens for quality control.

Statistical analysis

Chi-square test was used to determine the significance of differences of resistance prevalence between stray dogs and hospitalized dogs. A value of $p \leq 0.05$ was considered significant.

Results

A total of 628 *E. coli* isolates were recovered from 877 intestinal samples of stray and hospitalized companion dogs analyzed in this study: 422 isolates from stray dogs and 206 isolates from hospitalized dogs. The resistance to 15 antimicrobial agents for *E. coli* isolates from both groups of dogs is shown in Table 1. In general, most common resistance in *E. coli* isolates from both groups was to tetracycline, streptomycin, ampicillin, nalidixic acid, and trimethoprim/

Table 1. Antimicrobial Resistance Among *Escherichia coli* Isolates from Pet Dogs During 2006–2007 in Korea

Antimicrobials	*Concentration disk (μg)*	*Diffusion zone breakpoint (mm)*	*Resistance % (no. of resistant isolates)*		
			Stray dogs (n = 422)	*Hospitalized dogs (n = 206)*	*Total (n = 628)*
Ampicillin	30	≤13	32.9 (139)	47.1 (97)	37.6 (236)
Amoxicillin/clavulanic acid	20/10	≤13	5.2 (22)	6.3 (13)	5.6 (35)
Nalidixic acid[a]	30	≤13	21.6 (91)	37.4 (77)	26.8 (168)
Ciprofloxacin	5	≤15	13.5 (57)	21.4 (44)	16.1 (101)
Cephalothin	30	≤14	8.5 (36)	18.4 (38)	11.8 (74)
Cefoxitin	30	≤14	4.7 (20)	4.4 (9)	4.6 (29)
Cefotaxime	30	≤14	2.4 (10)	3.9 (8)	2.9 (18)
Cefepime	30	≤14	0.7 (3)	0 (0)	0.5 (3)
Imipenem	10	≤13	0 (0)	0 (0)	0 (0)
Amikacin	30	≤14	0.5 (2)	1.5 (3)	0.8 (5)
Streptomycin	10	≤11	35.8 (151)	41.7 (86)	37.7 (237)
Gentamicin	10	≤12	16.1 (68)	21.8 (45)	18 (113)
Chloramphenicol	30	≤12	17.1 (72)	24.3 (50)	19.4 (122)
Trimethoprim/sulfamethoxazole[a]	1.25/23.75	≤10	19.7 (83)	36.4 (75)	25.2 (158)
Tetracycline	30	≤11	53.6 (226)	52.4 (108)	53.2 (334)

[a] $p < 0.05$.

sulfamethoxazole. *E. coli* isolated from hospitalized dogs showed higher resistance to most of antimicrobials tested in this study compared with those isolated from stray dogs, although significantly higher resistance observed in hospitalized dogs was only against nalidixic acid and trimethoprim/sulfamethoxazole ($p < 0.05$). No resistance against imipenem was observed in *E. coli* isolates from both groups of dogs. No resistance against cefepime, a fourth-generation cephalosporin, was observed in *E. coli* isolated from hospitalized dogs. However, 1 (0.2%) and 3 (0.7%) isolates from stray dogs showed resistance against these two antimicrobial agents, respectively. Meanwhile, resistance against fluoroquinolone and gentamicin was relatively common in both groups of dogs, although the prevalence was much higher in hospitalized dogs than in stray dogs: resistance against ciprofloxacin and gentamicin reached 16.1% (13.5–21.4%) and 18% (16.1–21.8%), respectively. Some of the isolates from both groups of dogs showed resistance to cefotaxime (2.4–3.9%), a third-generation cephalosporin. Amikacin resistance was also observed in two (0.5%) and three (1.5%) isolates from stray and hospitalized dogs, respectively.

Antimicrobial resistance frequency among *E. coli* isolates from dogs by age group ($n = 628$) is presented in Table 2. *E. coli* isolates from younger dogs have significantly higher frequency of resistance against most of the antimicrobials tested in this study ($p < 0.5$), compared with isolates from older dogs.

The phenotypes of resistance exhibited by the 422 *E. coli* isolates from stray dogs and 206 isolates from hospitalized dogs are presented in Table 3. About 39% (162/422) and 27% (55/206) of the *E. coli* isolates from stray dogs and hospitalized dogs showed susceptibility to all antimicrobials tested in this study. The percentage of strains showing multiresistance (resistance to three or more subclasses of antimicrobial agents) was 32% and 48% of *E. coli* isolates from stray dogs and hospitalized dogs, respectively. Although tetracycline resistance was the most frequently observed among *E. coli* isolates (13.7%) from stray dogs, combined resistance to tetracycline–nalidixic acid–ciprofloxacin–chloramphenicol–ampicillin–aminoglycosides was the most common among isolates (8.3%) from hospitalized dogs. However, no significant difference was observed in the patterns of multiresistance between the isolates from stray dogs and hospitalized dogs.

Discussion

In general, *E. coli* isolated from both groups of dogs showed high percentages of resistance to ciprofloxacin, gentamicin, cefotaxime, or amikacin, compared with previous reports from European countries such as Portugal,[4] Finland,[18] and Denmark.[17] The most common resistance observed in this study was to tetracycline, followed by streptomycin, ampicillin, nalidixic acid, and trimethoprim/sulfamethoxazole. This finding is in agreement with the results of previous studies,[4,17] which have shown a common occurrence of resistance to these antimicrobials in *E. coli* isolates from both healthy and treated dogs, although the prevalence of resistance in this study is much higher than those of the European studies. The high frequency of resistance to these antimicrobials in dogs may be associated with the amounts of their usage in animals, since they are mostly used as the first-line antimicrobials in the treatment of common infections such as respiratory infections in dogs and cats.[7] According to Korea Animal Health Products Association,[12] the most common antimicrobials used in animal hospitals, including large and small animal clinics, were cephalexin, oxytetracycline, streptomycin, penicillin, chloramphenicol, and ampicillin in this order in 2005. A possible association between antimicrobial use and emergence of antimicrobial resistance in pet animal was also documented by some authors.[3,8]

Although limited data are available for comparison, bacterial isolates from treated dogs were more resistant than those from untreated dogs.[17,18] Similarly, the frequency of resistance was higher in isolates from hospitalized dogs than

Table 2. Antimicrobial Resistance Among *Escherichia coli* Isolates from Pet Dogs by Age Group ($N = 628$)

	Resistance % (no. of resistant isolates)[a]				
Antimicrobials	*<6 month (n = 98)*	*7 month–2 years (n = 266)*	*3–7 years (n = 181)*	*>8 years (n = 34)*	*Unknown (n = 49)*
Ampicillin	42.9 (42)	36.8 (98)	32.6 (59)	38.2 (13)	49 (24)
Amoxicillin/clavulanic acid	5.1 (5)	6.4 (17)	4.4 (8)	5.9 (2)	6.1 (3)
Nalidixic acid	31.6 (31)	26.7 (71)	21.5 (39)	23.5 (8)	38.8 (19)
Ciprofloxacin	19.4 (19)	17.7 (47)	12.7 (23)	11.8 (4)	16.3 (8)
Cephalothin	13.3 (13)	10.2 (27)	11.6 (21)	5.9 (2)	22.4 (11)
Cefoxitin	4.1 (4)	5.6 (15)	3.3 (6)	2.9 (1)	6.1 (3)
Cefotaxime	5.1 (5)	2.3 (6)	2.2 (4)	2.9 (1)	4.1 (2)
Cefepime	0 (0)	0.8 (2)	0.6 (1)	0 (0)	0 (0)
Imipenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Amikacin	4.1 (4)	0 (0)	0.6 (1)	0 (0)	0 (0)
Streptomycin	45.9 (45)	38.3 (102)	33.1 (60)	26.5 (9)	42.9 (21)
Gentamicin	26.5 (26)	19.5 (52)	12.7 (23)	11.8 (4)	16.3 (8)
Chloramphenicol	19.4 (19)	19.9 (53)	18.2 (33)	11.8 (4)	26.5 (13)
Trimethoprim/sulfamethoxazole	28.6 (28)	27.1 (72)	19.3 (35)	23.5 (8)	30.6 (15)
Tetracycline	57.1 (56)	53.8 (143)	52.5 (95)	44.1 (15)	51 (25)

[a] $p < 0.05$.

Table 3. Phenotypes of Resistance Among *Escherichia coli* Isolates from Pet Dogs During 2006–2007 in Korea ($N = 628$)

	Stray dogs		Hospitalized dogs	
No. of antimicrobials	*No. of isolates (%)*	*Most frequent pattern (%)*	*No. of isolates (%)*	*Most frequent pattern (%)*
Pan-susceptible	162 (38.4)	—	55 (26.7)	—
1	78 (18.5)	TE (13.7)	33 (16.0)	TE (6.3)
2	45 (10.7)	TE, AG (6.2)	19 (9.2)	TE, AG (2.4)
3	33 (7.8)	TE, AM, AG (4.5)	19 (9.2)	TE, AM, AG (1.9)
4	17 (4.0)	TE, CM, AM, AG (1.4)	11 (5.3)	TE, SXT, AM, AG (2.9)
5	31 (7.3)	TE, SXT, CM, AM, AG (2.4)	20 (9.7)	TE, NA, SXT, AM, AG (2.9)
6	17 (4.0)	TE, NA, SXT, CM, AM, AG (2.1)	21 (10.2)	TE, NA, CIP, CM, AM, AG (8.3)
7	19 (4.5)	TE, NA, SXT, CIP, CM, AM, AG (2.6)	14 (6.8)	TE, NA, SXT, CIP, CM, AM, AG (3.4)
8	5 (1.2)	TE, NA, SXT, CIP, CF, CM, AM, AG (0.9)	7 (3.4)	TE, NA, SXT, CIP, CF, CM, AM, AG (2.4)
9	3 (0.7)	TE, NA, SXT, CIP, CF, FOX, AM, AmC, AG (0.5)	6 (2.9)	TE, NA, SXT, CIP, CF, CTX, CM, AM, AG (1.5)
10	7 (1.7)	TE, NA, SXT, CIP, CF, FOX, CM, AM, AmC, AG (1.7)	1 (0.5)	TE, NA, SXT, CIP, CF, FOX, CM, AM, AmC, AG (0.5)
11	5 (1.2)	TE, NA, SXT, CIP, CF, FOX, CTX, CM, AM, AmC, AG (1.4)		
Total	422		206	

AM, ampicillin; AmC, amoxicillin/clavulanic acid; CF, cephalothin; FOX, cefoxitin; CTX, cefotaxime; CIP, ciprofloxacin; NA, nalidixic acid; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; AG, aminoglycosides; CM, chloramphenicol.

those from stray dogs against most antimicrobials tested in this study. Since the isolates included in this study were originated from samples submitted by stray dog shelters and small animal clinics throughout the country, they may be considered to be representative and epidemiologically unrelated. However, stray dogs tested in this study could not be simply assumed to be healthy dogs, because no health status of each stray dog was available at the time of sample. Therefore, studies should be carried out in the future to obtain more data on antimicrobial resistance among both pathogenic and indicator bacteria from companion animals including healthy dogs.

When compared to other livestock animals in Korea, the prevalence of resistance observed in this study was generally higher than in healthy cattle but lower than in healthy pigs and poultry.[9,14] Especially, resistance against antimicrobials commonly used as feed additives or used for a long time in livestock animals such as tetracycline, ampicillin, and streptomycin was much lower in both groups of dogs than in pigs and poultry: 52.4–53.6%, 32.9–47.1%, and 35.8–41.7% in dogs, whereas 96.3%, 66.1%, and 66.8%[14] or 99.2%, 75.2%, and 85.7%[9] in pigs and 80.4%, 63.8%, and 85.7%[9] in poultry, respectively. However, *E. coli* isolates from both groups of dogs showed higher resistance against antimicrobials that are used for treatment such as ciprofloxacin (13.5–16.1%), cephalothin (8.5–18.4%), and gentamicin (16.1–21.8%), compared with 7.8%, 7.3%, and 10.2% from pigs,[14] respectively. Nonetheless, resistance against ciprofloxacin (13.5–16.1%), sulfamethoxazole, and trimethoprim (19.7–36.4%) was still much lower in dogs, compared with 50.1%, 92.0%, and 69.9% in poultry,[9] respectively.

E. coli isolates from younger dogs have significantly higher resistance than the isolates from older dogs in this study. Evidence that young animals harbor a more resistant enteric flora than older animals has been reported by many researchers, although most of those studies were conducted on cattle.[10,15] In a previous study, however, *E. coli* isolates from older dogs showed higher resistance compared with isolates from younger dogs,[18] which is contrary to our result. To date, few studies have investigated the effect of age on resistance in dogs and more studies are needed in the future.

In this study, patterns of multiresistance in *E. coli* isolates from both stray dogs and hospitalized dogs were similar. Also, about 3% (12/422) of the isolates from stray dogs showed resistance to more than 10 out of 16 antimicrobials tested, whereas only one isolate (0.5%) from hospitalized dogs was resistant to 10 antimicrobials. A previous study reported that the prevalence of resistance and multiresistance in *E. coli* strains from kennel dogs was significantly higher than those observed in strains originating from individually held dogs.[5] The authors also documented that the reason for the elevated resistance prevalence in strains from kennel dogs was probably group housing. When a stray dog was found and delivered to a stray dog shelter, it remains there for a certain period. Then, the animals are adopted or mostly euthanized. During the holding period in shelters, stray dogs could have been exposed to bacteria from cohabitated dogs, which may have contained resistance genes that could be transmitted horizontally among dogs in the same place. Further work is needed to confirm this.

In conclusion, *E. coli* isolated from stray pet dogs and hospitalized pet dogs in Korea showed higher resistance to

most antimicrobials tested, compared with isolates from dogs in European countries. Also, some isolates showed resistance against antimicrobials that are regarded as critically important for use in humans such as third- and fourth-generation cephalosporins and quinolones. This finding indicates that more attention should be paid to the use of antimicrobials and the occurrence of antimicrobial resistance in companion animals. To our knowledge, this is the first report of antimicrobial resistance in fecal *E. coli* isolates from companion dogs in Korea.

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Disclosure Statement

No competing financial interests exist.

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