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Antimicrobial Resistance in *Escherichia coli* from Hospitalized and Kennel Dogs by Agar Disc Diffusion (Bauer-Kirby) Test

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ABSTRACT

Rectal swabs were collected from fifteen hospitalized and twenty kennel dogs. At sampling time, the hospitalized dogs were treated with different antimicrobial agents. None of the kennel dogs had been treated with an antimicrobial agent at least two months before sampling. *Escherichia coli* colonies were purified on blood agar and McConkey agar and confirmed by biochemical tests. Twenty seven samples were designated as *E. coli*. Eleven *E. coli* isolates originated from the hospitalized dogs and sixteen from the kennel dogs. After that, nine antimicrobial drugs which were amoxicillin, ampicillin, amoxicillin/clavulanic acid, cephazolin, ceftriaxone, enrofloxacin, oxytetracycline, gentamicin, and azithromycin were tested for antimicrobial sensitivity.

In the hospitalized dogs, the isolates were resistance to azithromycin, amoxicillin, oxytetracycline, ampicillin, cephazolin, and enrofloxacin at 81.81, 72.72, 63.63, 63.63, 54.55, and 54.55%, respectively, while susceptibility to antimicrobial drugs were consecutively found on gentamicin, ceftriaxone and amoxicillin/clavulanic acid. *E. coli* isolates from kennel dogs were particularly resistant to azithromycin, oxytetracycline, and amoxicillin at 68.75, 56.25, and 43.75%, consecutively and most isolates were susceptible to ceftriaxone, gentamicin, enrofloxacin, and cephazolin. In this study, the percentage of *E. coli* isolates that were resistant to all antimicrobial agents was higher in the hospitalized dogs than in the kennel dogs. The correlation between types of antimicrobial agents which dogs have received and the percentage of resistant *E. coli* isolates were also observed in this study.

Key words: antimicrobial resistance, *Escherichia coli*, hospitalized dogs, kennel dogs

INTRODUCTION

Nowadays, pet animal numbers have risen and become more meaningful to human community. For effective management to cure infections, antibiotic drugs are widely used in small animals. However, the bacterial sensitivity test is rarely performed for the initial treatment because it is time consuming and inconvenient. Thus, the new generation of broad-spectrum antibiotics such as aminopenicillins plus clavulanic acid, cephalosporins and fluoroquinolones are generally used for treatment (Guardabassi *et al.*, 2004). The more extensive use of antibiotic drugs without regulations and policies in companion animals especially dogs and cats, the antimicrobial-resistant problem has substantially increased. On the other hand, the current policies on

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antimicrobial usage in livestock are restricted in order to limit the antimicrobial-resistant problem in food animals (Guardabassi *et al*., 2004).

Not only is there concern about the antimicrobial resistance in animal and human medicine, there is also growing concern about the transmission of antimicrobial resistant bacteria between human and animal (Johnson *et al*., 2001; Moyaert *et al*., 2006) because of the close contact between owners and their pets. Several studies indicated that the transmission of antimicrobial resistant bacteria is suspected (Weese *et al*., 2006). Additionally, the prevalence of multiple drug resistance (MDR) in some types of bacteria isolated from canine and feline patients has expanded (Normand *et al*., 2000; Trott *et al*., 2004). Hence, it seems essential to be aware of an antimicrobial resistant trend in pet animals.

Escherichia coli, the intestinal flora of dogs, is one of the indicator bacteria to evaluate the antimicrobial-resistant level (Normand *et al*., 2000; De Graef *et al*., 2004). *E. coli* is usually isolated from the infected surgical wound and lower urinary tract infections (Carattoli *et al*.,2005). Pathogenic strains of *E. coli* can cause serious diseases and nosocomial illnesses in veterinary hospitals or clinics (Sanchez *et al*., 2002).

The objective of this study was to investigate the extent of antimicrobial-resistance *E. coli* in two populations of dogs. This information is very useful because veterinary clinicians will realize the antimicrobial-resistance trend that helps them choose the appropriate drugs for treatment and is also important for studying the transmission of antimicrobial-resistance bacteria between pets and human in the future.

MATERIALS AND METHODS

1. Sampling

Rectal swabs were collected from fifteen hospitalized and twenty kennel dogs. At sampling time, hospitalized dogs from the Veterinary Teaching Hospital, Kasetsart University, were receiving with different antimicrobial agents but kennel dogs had not been treated with an antimicrobial agent at least two months before sampling.

Skin around the anus was cleaned with 70% ethanol before sampling. Then each sterile cotton swab was inserted individually 2 cm into the anus. After that, the swab was kept in modified Stuart medium at 4°C and brought to the microbiology laboratory at the Faculty of Veterinary Technology, Kasetsart University for bacterial cultivation within 6 hours.

2. Bacterial identification

Isolation of bacteria

Each swab was inoculated on McConkey agar and incubated at 37°C for 16-18 hours. Then two suspected *E. coli* colonies were purified on blood agar and McConkey agar. All plates were incubated at 37°C for 16-18 hours. Purified colony of each plate was picked up for Gram stain and biochemical tests.

Biochemical test

One suspected *E. coli* colony of each plate was tested for biochemistry by motility, indole, lysine decarboxylase, citrate utilization and Triple sugar iron (TSI). Presumed *E. coli* colonies were tested for antimicrobial susceptibility.

3. Antimicrobial susceptibility test: disk diffusion method

E. coli isolates were picked up to suspend in phosphate buffer saline (PBS) as the similar turbidity as McFarland No.0.5. Then sterile cotton swabs were dipped and spread the suspension onto Mueller Hinton Agar (MHA).

After that, nine antimicrobial drugs which were amoxicillin (10μg/disc), ampicillin (10μg/disc), amoxicillin/clavulanic acid 2:1 (30 μg/disc), cephazolin (30 μg/disc), ceftriaxone (30 μg/disc), enrofloxacin (5 μg/disc), oxytetracycline (30 μg/disc), gentamicin (10 μg/disc) and azithromycin (15μg/disc) were placed on the spread MHA plates. All the plates were incubated at 37°C for 16-18 hours. Each isolate was classified as sensitive or resistant by the inhibition zone diameters surrounding the discs as shown on Table 1.

RESULTS

1. Isolation and identification of *E. coli*

Totally, 35 suspected *E. coli* (lactose-positive on McConkey agar) were purified on blood agar. After being tested by biochemistry, twenty seven of them were designated as *E. coli*. Eleven isolates originated from hospitalized dogs and sixteen from kennel dogs. The records of current therapeutics and the history of using antibiotic drugs in hospitalized and kennel dogs are shown in Tables 2 and 3, respectively.

2. Antimicrobial susceptibility tests

The result of antimicrobial susceptibility test to *E. coli* isolates from eleven hospitalized dogs and sixteen kennel dogs are shown in Tables 4 and 5.

The percentage comparison of each antibiotic drug sensitivity that are classified as resistant, intermediate and susceptible between hospitalized and kennel dogs are presented in Table 6. Considering the percentage of resistant, hospitalized dogs were more resistant than kennel dogs in all the antibiotic drugs used.

For the hospitalized dogs, resistance to azithromycin, amoxicillin, oxytetracycline, ampicillin, cephazolin and enrofloxacin was found at 81.81, 72.72, 63.63, 63.63, 54.55 and 54.55%, respectively, while susceptibility to antimicrobial drugs was consecutively found on gentamicin, ceftriaxone and amoxicillin/clavulanic acid at 72.72, 63.63 and 54.55%.

In the kennel dogs, resistance to azithromycin, oxytetracycline and amoxicillin were at 68.75, 56.25 and 43.75%, respectively. As for susceptibility to antimicrobial drugs, ceftriaxone, gentamicin, enrofloxacin and cephazolin, it was found at 100, 87.5, 87.5 and 87.5%, respectively.

DISCUSSION

In both of hospitalized and kennel dogs, the most isolates were resistant to azithromycin. It may have resulted from the mutation of *E. coli* (Hansen *et al.*, 2002). A high frequency of resistance to oxytetracycline was observed. Similarly, Costa *et al.* (2007) reported that almost 20% of the isolates in their study had shown

Table 1 Zone size interpretation chart.*

Antibiotic disc (μg/disc)	Zone diameter (mm.)		
	Resistance	Intermediated	Susceptibility
Amoxicillin (10)	≤13	14-16	≥17
Ampicillin (10)	≤13	14-16	≥17
Amoxicillin/clavulanic acid (30)	≤13	14-17	≥18
Cephazolin (30)	≤14	15-17	≥18
Ceftriaxone (30)	≤13	14-20	≥21
Enrofloxacin (5)	≤17	18-20	≥21
Oxytetracycline (30)	≤14	15-18	≥19
Gentamicin (10)	≤12	13-14	≥15
Azithromycin (15)	≤13	14-17	≥18

* = modified from NCCLS, 1999

tetracycline resistance.

When considering the percentage of resistance in hospitalized dogs, high levels of resistance to amoxicillin and ampicillin may have developed because these antibiotics were frequently used in the past and these dogs might have received them before being treated in this animal hospital. Hence, the incidences of β-lactamase-producing resistant organisms, including *E. coli*, appear to be increasing. In our study, *E. coli* was found to be resistant to enrofloxacin and cephazolin, the first generation of cephalosporin which is widely used nowadays (Guardabassi *et al.*, 2004). Oppositely, *E. coli* isolates from this study were highly susceptible to amoxicillin/clavulanic acid, ceftriaxone and gentamicin. Amoxicillin/clavulanic acid and ceftriaxone, the third generation of cephalosporin, are new generation of antibiotic drugs discovered to solve the resistance problem and both of them

Table 2 The record of using antibiotic drugs in hospitalized dogs.

Number	Date (mm/yy)	Antibiotic drugs
H1	01/07	Cephalexin
	01/07	Amoxicillin-clavulanic acid
H2	01/07	Cephalexin
H3	01/07	Enrofloxacin
	01/07	Cephalexin
H4	11/06	Cephalexin
	01/07	Cephalexin
	01/07	Enrofloxacin
H5	01/07	Amoxicillin-clavulanic acid
H6	01/07	Cephalexin
H7	04/99	Amoxicillin
	10/06	Doxycycline
	11/06	Cephalexin
	11/06	Enrofloxacin
	11/06	Amoxicillin-clavulanic acid
	11/06	Azithromycin
	12/06	Gentamicin
	12/06	Disento
	01/07	Amoxicillin
H8	01/07	Enrofloxacin
	01/07	Amoxicillin
H9	12/06	Cephalexin
	01/07	Cephalexin
H10	01/07	Enrofloxacin
	01/07	Oxytetracycline
H11	12/06	Cephalexin

Table 3 The record of using antibiotic drugs in kennel dogs.

Number	Date (mm/yy)	Antibiotic drugs
K1	N	N
K2	12/05	Penicillin G
K3	11/06	Amoxicillin
K4	05/06	Penicillin G
K5	N	N
K6	N	N
K7	05/06	Amoxicillin
	06/06	Amoxicillin
	07/06	Amoxicillin
K8	N	N
K9	N	N
K10	05/06	Enrofloxacin
K11	12/04	Penicillin G
	02/06	Penicillin G
K12	12/04	Sulfa-trimetroprime
	08/05	Amoxicillin
K13	10/04	Penicillin-Streptomycin
	11/04	Penicillin-Streptomycin
	10/05	Penicillin G, Amoxicillin
K14	11/06	Amoxicillin
K15	03/06	Enrofloxacin
	08/06	Amoxicillin
K16	08/06	Amoxicillin

N = Not receive any antibiotic drugs during sampling are substantially used. If veterinarians still use these antibiotic drugs without concern, the antimicrobial resistance levels will be greater in the near future. The most isolates in this study were susceptible to gentamicin, which is a narrow-spectrum antibiotic drug for Gram negative bacteria and is less used than the others because of its side effects and inconvenient for treatment. For the kennel dogs, which were usually treated with beta-lactam antibiotic such as amoxicillin and penicillin G. *E. coli* isolates were extremely resistant to amoxicillin and ampicillin, whereas a

Table 4 The result of individual antimicrobial susceptibility test.

ABO / type	OT	CN	AML	AMP	AMC	ENR	AZM	CRO	KZ
Hospitalized dogs									
H1	R	S	R	R	S	R	R	S	S
H2	R	R	R	R	R	R	R	I	R
H3	S	S	S	S	S	*	R	S	R
H4	I	S	S	S	S	S	R	S	S
H5	R	S	R	R	S	R	R	S	S
H6	*	S	S	S	S	S	I	S	S
H7	R	R	R	R	R	R	R	R	R
H8	R	S	R	R	I	R	I	S	R
H9	R	S	R	R	R	S	R	I	R
H10	R	R	R	R	I	R	R	R	R
H11	*	S	R	S	S	S	R	S	S
Kennel dogs									
K1	S	S	S	S	S	S	I	S	S
K2	R	R	R	R	I	S	I	S	S
K3	R	S	I	S	S	I	R	S	S
K4	R	S	S	S	S	S	I	S	S
K5	S	S	R	S	R	R	R	S	S
K6	S	S	S	S	S	S	R	S	S
K7	R	S	S	S	S	S	I	S	R
K8	*	S	I	S	S	S	R	S	S
K9	R	S	R	R	I	S	R	S	R
K10	S	S	*	R	I	S	R	S	S
K11	S	*	S	S	S	S	R	S	S
K12	R	S	I	I	I	S	R	S	S
K13	I	S	R	R	S	S	R	S	S
K14	R	S	R	R	S	S	R	S	S
K15	R	S	R	S	I	S	I	S	S
K16	R	S	R	R	R	S	R	S	S

* = can not interpret
S = Susceptible; I = Intermediate; R = Resistant
ABO = Antibiotic; OT = Oxytetracycline; CN = Gentamicin; AML = Amoxicillin;
AMP = Ampicillin; AMC = Amoxicillin-clavulanic acid; ENR = Enrofloxacin;
AZM = Azithromycin; CRO = Ceftriaxone; KZ = Cephazolin

Table 5 The result of antimicrobial susceptibility test.

Antibiotic drugs	Hospitalized dogs (sample)			Kennel dogs (sample)		
	R	I	S	R	I	S
Oxytetracycline	7	1	1	9	1	5
Gentamicin	3	0	8	1	0	14
Amoxicillin	8	0	3	7	3	5
Ampicillin	7	0	4	6	1	9
Amoxicillin/clavulanic acid 2:1	3	2	6	2	5	9
Enrofloxacin	6	0	4	1	1	14
Azithromycin	9	0	2	11	0	5
Ceftriaxone	2	2	7	0	0	16
Cephazolin	6	0	5	2	0	14

S = Susceptible; I = Intermediate; R = Resistant

Table 6 The percentage comparison of antimicrobial susceptibility test.

Antibiotic drugs	Resistant (%)		Intermediated (%)		Susceptibility (%)	
	H	K	H	K	H	K
Oxytetracycline (OT)	63.63	56.25	9.09	6.25	9.09	31.25
Gentamicin (CN)	27.27	6.25	0	0	72.72	87.5
Amoxicillin (AML)	72.72	43.75	0	18.75	27.27	31.25
Ampicillin (AMP)	63.63	37.5	0	6.25	36.36	56.25
Amoxicillin/clavulanic acid 2:1 (AMC)	27.27	12.5	18.18	31.25	54.55	56.25
Enrofloxacin (ENR)	54.55	6.25	0	6.25	36.36	87.5
Azithromycin (AZM)	81.81	68.75	18.18	31.25	0	0
Ceftriaxone (CRO)	18.18	0	18.18	0	63.63	100
Cephazolin (KZ)	54.55	12.5	0	0	45.45	87.5

H = *E. coli* isolates from hospitalized dogs; K = *E. coli* isolates from kennel dogs

relatively high frequency of isolates were susceptible to the other antibiotic drugs except oxytetracycline. Likewise, the study of Costa (2007) showed that *E. coli* from healthy pets were highly resistant to ampicillin. This was related to the frequency and types of antibiotic drugs which they used to receive. Four *E. coli* isolates from kennel dogs, which used to obtain amoxicillin (K3, K7, K12, K13, K14, K15, and K16) were resistant to this type of drug. Several dogs (K1, K5, K6, K8, and K9) have never obtained any antibiotic drugs before sampling, but *E. coli* from K5, K6, K8, and K9 was not susceptible to all antibiotic drugs. This result may infer that not only the frequency and types of antibiotic drugs, the transmission of these bacteria among dogs in the same house affected to increase antimicrobial resistance bacteria. Moreover, *E. coli* isolates from three hospitalized dogs (H2, H7, and H10) were not susceptible to any antibiotic drugs. One of them (H7) obviously received many types of antibiotic drugs within 3 months but the others were treated with fewer types of drug. Thus, the transmission of antimicrobial resistance bacteria should be considered and also awareness of nosocomial infection. In addition, the study of Boerlin *et al.* (2001) demonstrated that the potential for hospital nosocomial resistance problems in veterinary medicine was similar to those encountered in human medicine. However, the transmission of antimicrobial resistance bacteria between pets to owners is doubted (Weese *et al*., 2006), it should be verified in the future to prevent this phenomenon.

Further study should enhance the sample size and isolate more diverse types of indicator bacteria including Gram positive bacteria which can provide valuable information about the current antimicrobial resistance tendency and the transmission of antimicrobial resistance bacteria in companion animals.

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