Research article

Antimicrobial Resistance Profiles Found in a Case Study of *Escherichia coli* from Cohabitant Pets and Environmental Surfaces at Animal Clinics, Phnom Penh City

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Abstract *Escherichia coli* (*E. coli*) can be transmitted from dogs to surfaces through direct exposure to dog faeces, pus, and urine; alternatively, it can live in the environment for a short period. We aimed to determine the prevalence and antibiotic resistance profile of *E. coli* from dogs and environmental surfaces of animal clinics in Phnom Penh, Cambodia. We swabbed twenty-four samples from dogs (18 faeces and 6 pus) and twenty-three from environmental surfaces (8 cage-floors, 7 cage-walls, 1 feeding-plate, and 7 treatment-tables) at animal clinics. The bacterial culture method was used to isolate *E. coli* from both dog and environmental surface samples. An antibiogram of the isolates was tested using the disk diffusion method with six antibiotics (amoxicillin, ampicillin, ceftriaxone, gentamycin, levofloxacin, and tetracycline), following the standard of the Clinical and Laboratory Standard Institute. The results showed that *E. coli* was present in approximately 54% of dog samples and 22% of environmental surface samples. The *E. coli* isolates from dog samples showed high resistance to amoxicillin, ampicillin, and tetracycline (54%) and lower resistance to ceftriaxone, gentamycin (27%), and levofloxacin (8%). *E. coli* isolates from environmental surfaces demonstrated high resistance to amoxicillin, ampicillin, ceftriaxone, and tetracycline (80%-100%), and lower resistance to gentamicin and levofloxacin (60%). In conclusion, *E. coli* was found in both dogs and veterinary clinic surfaces and exhibited resistance to numerous antibiotics. This study suggests that further research is necessary to identify the specific genes responsible for the antibiotic resistance of *E. coli* found in dogs, on clinic surfaces, as well as in humans – including clinic technicians and dog owners – in order to better understand how this resistance is affected by the transmission of *E. coli* between different carriers.

Keywords dogs, environmental surfaces, *E. coli*, antibiotic resistance, antibiogram

INTRODUCTION

The use of antimicrobial drugs does not only increase the antimicrobial resistance (AMR) of pathogenic bacteria but also the endogenous commensal flora. (Berge et al., 2006; Dancer, 2004; Goosens, 2009). It is reported that the potential problem of AMR among pet animals may cause human health problems, due to the increasing utilization of the same antimicrobial substances in human medicine and to the close contact between pets and their human cohabitants (Guardabassi et al., 2004; Moyaert et al., 2006; Schwarz et al., 2001).

The spread of antimicrobial-resistant bacteria can occur directly, by skin-to-skin contact and contact with bacteria-containing material (e.g., saliva, feces, urine), or indirectly via the household environment (Guardabassi et al., 2004; Schwarz et al., 2001). Leite et al. (2013) reported that direct, close contact between all the cohabitants including pets and humans, and the touch of contaminated household surfaces and objects could lead to shared antimicrobial-resistant bacteria. In the new host, resistant bacteria can both colonize and infect and remain in that particular environment for a short time. During this period, the resistant bacteria are capable of either spreading their resistance genes to other bacteria residing in the new host (endogenous or pathogen bacteria), but also accepting resistance genes from other bacteria (Livermore, 2003; Schwarz et al., 2001). *E. coli* has possible biology in spreading resistance not only to the acceptor and donor of the transmissible drug-resistant gene but also commonly found in the intestinal tract of both humans and animals (Costa et al., 2008). *E. coli* can also be implicated in various intestinal and extra-intestinal diseases including urinary tract infection (Johnson et al., 2008; Johnson et al., 2001) and Pyometra (Bassessar et al., 2013). Likewise, Markey et al. (2013) showed that *E. coli* can cause diseases such as Colisepticaemia, Pyometra, and Urinary tract infections in dogs. No study has been conducted in Cambodia regarding the *E. coli* resistance in pets and its associated environment.

OBJECTIVE

The present study aimed to determine the prevalence and resistance profiles among *E. coli* from cohabitant pets and environmental surfaces at animal clinics in Phnom Penh City, Cambodia.

METHODOLOGY

Study Site and Sampling

The study was conducted in three animal clinics in Phnom Penh city, Cambodia from March to May 2019. In total, forty-seven samples were swabbed from dogs and environmental surfaces. The pet samples were selected based on the diagnosis of bloody diarrhea (3 to 8 months old), canine parvovirus, and Pyometra disease. The environmental samples were selected based on the case-reported animals above which animals were exposed to. Twenty-four samples (18 faeces and 6 pus) were collected from dogs. Twenty-three samples (8 cage-floors, 7 cage-walls, 1 feeding plate, and 7 treatment-tables) were collected from the environmental surface. All samples were collected and transported on the same day to the Veterinary Microbiology Laboratory of the Faculty of Veterinary Medicine, Royal University of Agriculture, Cambodia.

E. coli Isolation and Identification

Samples were collected and the bacterial culture was followed by Markey et al. (2013). Briefly, the swap was subcultured into 5 mL of Buffered Peptone Water (BPW) and incubated at 37^0C for 24 hours. Then the presumptive was streaked on MacConkey agar and incubated at 37^0C for 24 hours. The suspected colonies with large/medium dark pink colonies were confirmed with biochemical tests such as gram staining, Catala's test, Triple Sugar Iron (TSI), and Motility-Indole-Lysine (MIL) test.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disk diffusion method following Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines. Briefly, fresh *E. coli* isolates were plated on Trypton-Soja-Agar (TSA) plates and incubated at 37^0C for 24 hours. Then 2-3 colonies on TSA were inoculated on NaCl suspension to a turbidity equivalent to 0.5 McFarland standards. One hundred microliter suspension was plated on 15cm depth Mueller-Hinton agar (MHA) and standard discs were applied using a dispenser and incubated for 16-18 hours at 37^0C. A

total of six antimicrobial agents including Tetracycline (30 µg), Ampicillin (10 µg), Amoxicillin (10 µg), Gentamycin (10 µg), Ceftriaxone (30 µg), and Levofloxacin (5 µg) were tested. All antibiotics used for the study are based on the treatment protocol of each animal clinic.

RESULTS AND DISCUSSION

The present study found that a high prevalence of *E. coli* (54.16%) presented in dogs; whereas, a low prevalence (21.74%) presented in environmental surfaces isolated from the animal clinics in Phnom Penh city. *E. coli* presented in about 67% and 17% of dog faeces and pus, respectively. The result is similar to the previous findings of Das et al. (2012) on the faeces of dogs. *E. coli* is the major pathogen in the genital tract (Bassessar et al., 2013). Some studies have shown 50-85% prevalence of *E. coli* in bitches suffering from pyometra disease (Bjurström et al., 1993; Sandholm et al., 1975; and Trevena et al., 1996). Sharif et al. (2017) found that the prevalence of *E. coli* in the pus of bitches with pyometra was higher than the present study as the sample is much larger. Dog age is a factor contributing to the prevalence of *E. coli* in faeces. Greene et al. (1998) have reported two strains of *E. coli* were associated with gastrointestinal disease in young dogs. 77% of *E. coli* is present in young dogs at age 1-3 months (Younis et al., 2015). However, Torkan et al. (2016) showed a significantly lower prevalence of *E. coli* (47%) in the adult dog's faeces. The present study was on dogs aged 3-8 months and showed a prevalence somewhere between the high and low values reported by the aforementioned studies.

Table 1 The prevalence of *E. coli* distributed the animal clinics

Animal Clinics	Dogs (faeces and pus)		Environmental surface	
	Number (n/N)	Percent (%)	Number (n/N)	Percent (%)
A	1/5	20.00	0/2	0.00
B	5/10	50.00	5/16	31.25
C	7/9	77.78	0/4	0.00
Total	13/24	54.16	5/23	21.74

Table 2 The prevalence of *E. coli* distributed in animal and environmental surface samples

Sample		Prevalence		Total	
		Number (n/N)	Percent (%)	Number (n/N)	Percent (%)
Dogs	Faeces	12/18	66.67	13/24	54.16
	Pus	1/6	16.67		
Environmental surfaces	Cage-floor	3/8	37.50	5/23	21.74
	Cage-wall	1/7	14.28		
	Feeding plate	0/1	0.00		
	Treatment table	1/7	14.28		

Table 3 The antimicrobial resistance profiles of *E. coli* in dogs and environmental surfaces

Antibiotics	Dogs		Environmental surfaces	
	Number (n/N)	Percent (%)	Number (n/N)	Percent (%)
Tetracycline	7/13	53.85	5/5	100.00
Ampicillin	7/13	53.85	4/5	80.00
Amoxicillin	7/13	53.85	5/5	100.00
Gentamycin	3/13	23.08	3/5	60.00
Ceftriaxone	4/13	30.77	4/5	80.00
Levofloxacin	1/13	7.69	3/5	60.00

The prevalence of *E. coli* on the environmental surface varies based on the animals that have been exposed, according to Sidjabat et al. (2006). The highest prevalence of *E. coli* (37.50%) found on environmental surfaces of the animal clinics in this study was isolated from the cage floor, which is the most contaminated between faeces or pus of the dogs on the cage floor during hospitalization. Instead, it might also be attributed to poor hygiene of the animal clinics and the cross-contamination between dogs and the surfaces. Madubuike et al. (2016) reported a lower prevalence of *E. coli*

bacteria on environmental surfaces (3.60%), especially the cage floor (0%) of the animal clinics in Nigeria might be the biosecurity measures taken in the animal clinics were able to reduce the contamination of *E. coli*.

E. coli showed high resistance to amoxicillin, ampicillin, and tetracycline (54%); while less resistant to ceftriaxone (31%), gentamycin (23%), and levofloxacin (8%) in dogs. Similarly, these isolates also had high resistance to amoxicillin, ampicillin, ceftriaxone, and tetracycline (80-100%); while less resistance to gentamicin and levofloxacin (60%) on environmental surfaces. The high resistance to antibiotics on environmental surfaces was higher than the dog samples in the present study. This might be fewer samples in the environment and the possibility of *E. coli* surviving on the surfaces. Leite et al. (2013) and Carvalho et al. (2016) reported that pet dogs were shown to be a potential household source of multi-resistant *E. coli* strains to humans through the environment. Resistance to antibiotics in dogs in the present study is consistent with the study of Nam et al. (2010).

CONCLUSION

In conclusion, both dogs and environmental surfaces showed identical prevalence of *E. coli* and its resistance to several important antimicrobials. This is correlated with the spreading of antimicrobial resistance into the environmental surfaces of the animal clinics from hospitalized dogs and could be spreading to owners and veterinarians, a cause of concern with regard to animal and public health.

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