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Detection of Diarrheagenic *Escherichia Coli* in Pet Animals and Its Antibiotic Resistance in Alexandria Governorate

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ABSTRACT:

Benefits of having a dog or a cat as a pet varies between owners according to the purpose, however, the limit between benefit and harm is sensitive because close contact between pets and humans may involuntarily represent harm for humans. Dogs and cats have been proposed as a possible reservoir of virulent *Escherichia coli* strains that may cause enteric and extra-intestinal infections in humans. In this study, we aimed to detect diarrheagenic *Escherichia coli* (DEC) in dogs and cats and their antibiotic resistant pattern(s). Samples were collected from 70 dogs and cats from different veterinary clinics and hospitals in Alexandria. These animals suffered from diarrhea and other symptoms as fever, nausea, vomiting, chills, loss of appetite, muscle aches and bloating. Forty one *E. coli* positive samples were detected by culturing and biochemical tests, and were subjected to antimicrobial disc diffusion susceptibility test by using 10 different antibiotic discs, which are the most commonly used in pet animal clinics. Antibiotic resistance for individual antibiotics ranged from 5 to 98% with multiple resistances to 2 or more antibiotics detected. PCR for detection of virulent genes of *E. coli*; VT2e and eaeA genes as well as the antibiotic resistance bla_{TEM} gene was performed. The VT2e and eaeA genes were found in 20% and 60% of *E. coli* samples, respectively. These results collectively indicate that pet animals can harbor the *Enteropathogenic* (EPEC) and *Enterotoxigenic Escherichia coli* (ETEC) causing diarrhea at different ages with possible active transmission to contact human. Further, the high and multiple antibiotic resistance level can pose therapeutic challenges in contact humans. It is fundamental that veterinarians recommend preventive measures to pet owners towards the establishment of a long-term preventive programme against antibiotic resistant *E. coli*.

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1. INTRODUCTION

(*E. coli*) are widely distributed in the gut of humans and warm-blooded animals. Pathogenic strains of *E. coli* can cause gastrointestinal illness in healthy organisms (Feng et al., 2002). *E. coli* are colonized in the gastrointestinal tract within few hours after birth. Usually, commensal *E. coli* interact with the host in a mutually beneficial way; however, some strains of *E. coli* acquire virulence attributes that can cause a broad spectrum of disease. There are different classes of pathogenic *E. coli* strains which are responsible for outbreaks of diarrhea. These include *Enteropathogenic E. coli* (EPEC), *Enterohemorrhagic E. coli* (EHEC), *Enterotoxigenic E. coli* (ETEC), *Enetroaggregative E. coli* (EAEC), *Enteroinvasive E. coli* and *Diffusely adherent E. coli* (DAEC) (Sharma et al., 2006). EPEC and ETEC are the most causative agents of diarrhea in pet animals (Gracey et al., 1992).

Escherichia coli are usually susceptible to a variety of antibiotics, however with time and also due to the extensive use of antibiotics, drug resistant strains have evolved. Moreover, the production of extended spectrum β-lactamases (ESBL) producing enteric pathogens has been a serious issue (Mathur et al., 2002). With the extensive progress in the veterinary medicine filed and the increase in the number of pet animal clinics in Egypt, especially in Alexandria Governorate, the number of pets treated has increased and consequently increased the use of antimicrobial treatments. Unfortunately, the antimicrobials used are not restricted to those of veterinary use but also extended to the use of those used for human treatment. Further, this may favor the development of resistance strains of *E. coli* that can represent a zoonotic health hazard.

E. coli has been extensively studied in farm animals as well as pets around the world (Puño-Sarmiento et al., 2013) but little is known about its prevalence and antibiotic resistance pattern(s) in pet animals in Egypt. Here we aimed to study the prevalence of diarrheagenic *E. coli* in pet animals from different veterinary hospitals and clinics in Alexandria. Rectal swabs from dogs and cats at different ages were collected from animals suffering mainly from diarrhea and other symptoms as fever, nausea, vomiting, chills, loss of appetite, muscle aches and bloating. Antimicrobial susceptibility testing was carried out by the standard disk diffusion method according to guidelines established by the Clinical laboratory standards institute (CLSI, 2012). Further, some of the *E coli* positive samples were subjected to PCR for detection of *bla*$_{TEM}$ gene. The samples were further subjected to detect the most common virulence genes; eae*A* and VT2e by PCR.

2. MATERIAL AND METHODS

2.1. Animal samples and bacterial cultures:

A total of 70 rectal swabs were collected from dogs and cats at different ages from different veterinary hospitals and clinics in Alexandria. Fifty one samples of them were collected from dogs and 19 samples were collected from cats. The animals suffered mainly from diarrhea and other symptoms as fever, nausea, vomiting, chills, loss of appetite, muscle aches and bloating. Rectal swabs were cultivated onto nutrient broth for 18 h and then were inoculated onto MacConkey agar and Eosin Methylene Blue (EMB) agar and incubated aerobically at 37°C for 24 h. After 24 h incubation, the plates were observed for presence of bacterial colonies. Colonies from each sample were further subjected to Gram staining and biochemical testing as catalase test, urease test, IMViC and triple sugar iron agar (TSI) (MacFaddin, 1985).

2.2. Antimicrobial susceptibility testing:

Samples were cultured on MacConkey agar and Eosin Methylene Blue (EMB) agar and three colonies per sample were collected and then cultured onto nutrient agar for antimicrobial susceptibility testing, which was carried out by the standard disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI., 2012). The following antibiotic discs were used: cefotaxime (CTX), 30 µg; streptomycin(S), 10 µg; chloramphenicol(C), 30 µg; doxycycline (DO), 30 µg; Trimethoprim/Sulphamethoxazole (SXT), 25 µg; amikacin (AK), 30 µg; Amoxicillin/Clavulanic acid (AMC), 20\10 µg; gentamicin (CN), 10 µg; ciprofloxacin (CIP), 5 µg; erythromycin (E), 15 µg.

2.3. PCR detection of virulence and antibiotic resistance genes:

Three colonies per animal sample were collected and processed for detection of *E. coli* virulence genes. Search for virulence markers associated with E*nteropathogenic* and *Enterotoxogenic E. coli* was carried by PCR to detect both VT2e gene and *eaeA* gene, which are the most common virulence genes of *E. coli* in dogs and cats. The *eaeA* gene of *Enteropathogenic E. coli* (EPEC) is necessary for intimate attachment to epithelial cells. Also detection of *Escherichia coli* resistance gene; *bla*$_{TEM}$ gene was carried out (Coque et al.,2002).

Primers for amplifying segments of the VT2e, *eaeA*, and *bla*$_{TEM}$ genes are listed in Table (1).

Table (1): Oligonucleotide primers sequences used for PCR

Primer	Sequence (5'-3')	Product	Reference
eaeA	GACCCGGCACAAGCATAAGC	384 bp	Wen-jie *et al.*, 2008
	CCACCTGCAGCAACAAGAGG		
VT2e	CCAGAATGTCAGATAACTGGCGAC	322 bp	Orlandi *et al.*, 2006
	GCTGAGCACTTTGTAACAATGGCTG		
bla$_{TEM}$	ATCAGCAATAAACCAGC	516 bp	Colom *et al.*, 2003
	CCCCGAAGAACGTTTTC		

3. RESULTS

3.1. E. coli detection from dogs and cats

A total of 70 rectal swabs from 51 dogs and 19 cats at different ages were included in our study. These animals mainly suffered from diarrhea and other symptoms such as fever, nausea, vomiting, chills, loss of appetite, muscle aches and bloating. Samples were cultured for isolation of *E. coli* and subjected to biochemical tests. *E. coli* was isolated from 34 (66.6%) dogs and 7 (36.8%) cats. As shown in Table (2), *E. coli* isolation was higher in younger ages compared to older ones.

3.2. Antimicrobial susceptibility

Antimicrobial susceptibility was observed in all *E. coli* isolates from dogs and cats**.** Multiple antibiotic resistance was detected in all samples from dogs and cats. As shown in Table (3) and Fig. 2, high resistance levels were detected with the highest against Trimethoprim /Sulphamethoxazole (98%), erythromycin (97%) and cefotaxime (95%), while there was a high sensitivity to amikacin only. There was no correlation between the age of the animals and the single or multiple resistances.

3.3. virulence factors and antimicrobial resistance genes

Some of the *E. coli* positive samples, as shown in Table (4) were subjected to PCR for detection of the virulence factors VT2e and *eaeA*. The VT2e gene (ETEC) was found in *E. coli* isolated from dogs. The *eaeA* gene (EPEC) was found in *E. coli* isolated from dogs and cats.

Further, the samples were subjected to PCR for detection of bla_{TEM} gene. The results revealed that all tested samples were positive for the bla_{TEM} gene confirming the antimicrobial resistance pattern as shown in Fig.1

Table (2): Results of *E. coli* isolation from diarrheagenic dogs and cats at different ages.

	No. of examined samples		No. of *E. coli* positive cases		%	
	Dogs	Cats	Dogs	Cats	Dogs	Cats
≤1 - ≤ 3months	26	6	20	4	77	67
≤4 - ≤ 6months	10	5	6	2	60	40
≤7 - ≤ 3months	8	4	4	1	50	25
Over one year	7	4	4	0	57	0
Total	51	19	34	7	66.6	36.8

Table (3): Percentage of antibiotic resistance to *E coli*

Antibiotic	**% of resistance**
Amikacin (AK)	**5 %**
Erythromycin (E)	**97 %**
Chloramphenicol (C)	**88 %**
Cefotaxime (CTX)	**95 %**
Streptomycin (S)	**90 %**
Doxycycline (DO)	**88 %**
Trimethoprim/Sulphamethoxazole (SXT)	**98 %**
Amoxicillin/Clavulanic acid (AMC)	**88 %**
Gentamicin (CN)	**93 %**
Ciprofloxacin (CIP)	**90 %**

Table (4): *E. coli* virulence factors; VT2e and eae*A* in dogs and cats.

Animal	VT2e	*eaeA*
Dog	+	+
Dog	-	-
Cat	-	+
Dog	-	-
Dog	+	-

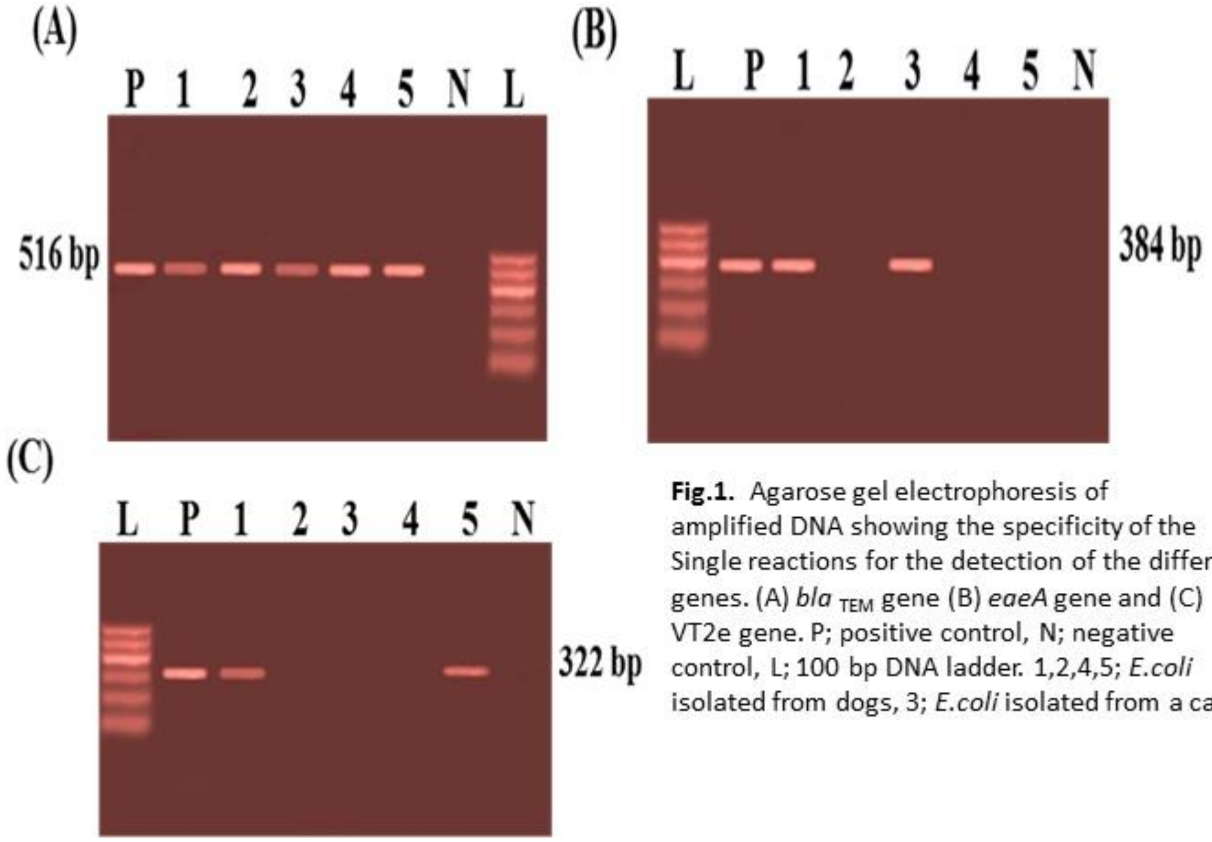

Fig.1. Agarose gel electrophoresis of amplified DNA showing the specificity of the Single reactions for the detection of the different genes. (A) *bla* $_{TEM}$ gene (B) *eaeA* gene and (C) VT2e gene. P; positive control, N; negative control, L; 100 bp DNA ladder. 1,2,4,5; *E.coli* isolated from dogs, 3; *E.coli* isolated from a cat.

4- DISCUSSION

Little is known about the role of pet animals in the transmission of *E. coli* to humans or even vice versa in Egypt. In our study, Samples were collected from dogs and cats at different ages, with total percentages of *E. coli* isolation of 58.5% (41/70). Dogs and cats from one to three months were the most susceptible to *E coli* infection; dogs represented 77% (20/26), while cats represented 67% (4/6). This agrees with Bores et al, 1988 who reported that *E. coli* was demonstrated in diarrheagenic puppies from one month to one month and half. Dogs and cats over one year were less susceptible to *E. coli* infection as shown in Table. (2). This agrees with several studies which had shown that the peak of incidence of enteritis was always in the few days after the beginning of the weaning period (1 month) and most of EPEC infections occur in the first 3 months of life as mentioned by Janke et al, 1989. However, in other studies in Trinidad (Adesiyun et.al., 1997), the prevalence of EPEC strains was not significantly associated with age.

EPEC and EPEC are the most common strains of *E. coli* in dogs and cats (Puño-Sarmiento et al., 2013). Some of our samples from dogs and cat were subjected to PCR for detection of virulence factors VT2e and *eaeA*. The VT2e gene was detected in *E. coli* samples from dogs, this agrees with what was mentioned by Abaas et al., 1989, who isolated VT2e from pet animals. The *eaeA* gene was found in *E. coli* isolated from dogs and cats. This agrees with what was mentioned by Jerse AE at al., 1990, who isolated *eaeA* gene from dogs and cats. Meanwhile, one dog was shown to harbor both virulence genes as shown in Table (4) indicating a possibility of mixed infection with two pathotypes of *E. coli*; EPEC and ETEC thus increasing the risk factor of zoonoses to close contact human. We believe that more animals can be harboring such mixed infection and therefore additional studies are required to resolve such issue.

Antimicrobial resistance in bacteria is a phenomenon that has been in constant evolution since the introduction of antibiotics. Several factors are known to promote bacterial resistance including: failure of following treatment regimen, prophylactic use of antibiotics, and the use of antibiotics as growth promoters as well as using antibiotics commonly used in human practice. This agrees with what was reported by Grave et al., 2006**.**

In our study, antimicrobial susceptibility to single as well as multiple antimicrobials was detected. Several samples even showed multiple resistances to several tested antimicrobials. Results in Table (3) showed high resistance against Trimethoprim/Sulphamethoxazole (98%), erythromycin (97%) and cefotaxime (95%), while there was a high sensitivity to amikacin only. This agrees with what was observed by Guardabassi et al., 2004. High sensitivity was only shown to amikacin, in contrast to the results of Walia et al. ,2004 who found that *E. coli* were sensitive to ampicillin, streptomycin and tetracyclin. Furthermore, Shehabi et al., 2004 showed resistance to gentamicin, ciprofloxacin and cefotaxime. These reports and the findings of the present study clearly indicate rapid and widening resistance patterns of E. coli. It may also imply that there is a possibility of acquisition of resistance E. coli in pet animals from human ones representing a bi-directional public health hazard (Patoli et al., 2010).

Most dog and cat owners seek the aid of a veterinarian only when their animals display overt disease signs. This is particularly true in developing countries, where most dog and cat owners care of round preventive programs. Naturally, in some rural and suburban areas the level of compliance may be even more reduced. However, a considerable number of pet owners are increasingly aware of the risks and potential costs attached to the non-adoption of preventive measures directed at avoiding the exposure to certain pathogens, such as *E. coli*. Thus, it is fundamental that veterinarians guide pet owners towards the establishment of long-term preventive programmes against infectious diseases as such caused by *E. coli*.

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