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Research Article

Occurrence of *Escherichia coli* as a Causative Agent of Enteritis in Dogs with Special Reference to Their Multidrug Resistance and Virulence Genes

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Abstract | *Escherichia coli* (*E. coli*) massively causes deaths all around the world. Human–pet proximity may inadvertently harm humans. Dogs are susceptible to virulent *E. coli* strains causing enteric infections in humans. In the present study, a total of 90 rectal swabs were collected from dogs of different ages (suffered from diarrhea with fever, nausea, chills, loss of appetite, and bloating) at different veterinary hospitals and clinics in Cairo to determine the incidence rate of *E. coli* and their virulence and resistance genes. Bacteriological examination revealed an overall *E. coli* occurrence rate of 67.7% (61/90). The highest isolation rate of 75.5% was from puppies that were 3 months and more. Serotyping of ten *E. coli* isolates showed that they belonged to seven serogroups O18, O27, O55, O126, O148, O158, and O166 and other untypable three strains. All *E. coli* isolates were subjected to disc diffusion sensitivity tests and were resistant to tetracycline, trimethoprim/ sulphamethoxazole (100% for each), cefotaxime (95.1%), and erythromycin (93.4%), while they were sensitive to amikacin only (88.5%). The occurrence of virulence genes in the seven tested *E. coli* isolates was conducted using PCR. It was revealed that the *stx1* gene was detected in O18, O126, O148, O158, and O166 serogroups while the *stx2* gene was detected in O18 and O27 only. The *eaeA* gene was detected in O27, O55, O148, and O158 serogroups. Also, the antibiotic-resistant *blaTEM* and *tetA* genes were detected in all the seven tested serotypes. These combined results indicate that pet animals may harbor *E. coli* causing diarrhea at different ages.

Keywords | Diarrhea, Dogs, Enteropathogens, *Escherichia coli*, Virulence genes, Shiga toxin, Drugs resistance.

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INTRODUCTION

Escherichia coli (*E. coli*) usually inhabits the gastrointestinal tract of animals; it can affect dogs, and humans. Also, it can be transmitted from animals to humans and vice versa. They are one of the most common enteric types present worldwide. Under certain conditions, these bacteria can also be responsible for causing diseases. Dogs and humans can be infected with *E. coli* by consuming contaminated water or food. *E. coli* usually affects weak animals, such as the too young, old, and malnourished animals or immune-compromised pets (Hustom, 2019). Canine puppies younger than one year old are quite prone to gastrointestinal infections, with acute diarrhea manifesting as the most usual clinical indicators; it could lead to severe dehydration and death (Hubbard et al., 2007).

Enterotoxigenic *E. coli*, for instance, is an *Escherichia coli* type that may induce foodborne sickness, beginning to manifest when compromised water or food is consumed. Enterotoxigenic *E. coli* emits a toxin that affects the inside of the infected intestinal tract, consequently causing diarrhea (Hustom, 2019). Virulence factors of pathogenic *E. coli* includes Shiga toxin-production (Croxen et al., 2013). Shiga toxins, *stx1* and *stx2* produced by the Shiga toxin-producing *E. coli* (STEC) strains are the chief virulence aspects. The strains possess the LEE (locus for the enterocyte effacement), where the *eae* gene resides. This

gene encodes for intimin, the adherence protein whose receptor is required for virulence (Ben Said et al., 2019). The ability of STEC to induce serious diseases correlates to the emittance of one or more Shiga-like toxins) *stx1*, s*tx2*, or other variants); they inhibit the host cells' synthesis of protein, leading to cell death (Ahsan et al., 2020).

Dogs that carry multidrug-resistant *E. coli* strain (MDREC) in their feces could contaminate the surrounding environment, as a result of transmitting these bacteria to humans as well as other animals (Warren et al., 2001). *E.* coli is often prone to various antibiotics; however, by time and antibiotics' excessive use, drug-resistant strains emerged. Moreover, extended-spectrum β-lactamases (ESBL) that induce enteric pathogens is a serious problem (Mathur et al., 2002).

This work aims to detect the occurrence rate of *E.coli* in diarrheic dogs of different ages, identify the isolates by VITEK2 compact, serological testing, and serogroup, as well as investigate the presence of the virulence (*stx1*, *stx2,* and *eae)* and drug resistance (*bla TEM* and *tet A*) genes.

MATERIALS AND METHODS

Samples Collection

A total of 90 rectal swabs were collected from dogs of different ages (one month to over one year) from different veterinary hospitals and clinics in Cairo. The animals were suffered from diarrhea with fever, bloating, chills, loss of appetite, and nausea. Each sample was collected using a sterile swab that was kept in an icebox immediately after collection and then sent to the laboratory without delay. All samples were taken with care about ethical guideline for sampling and also during working all biosafety measures were done. The experimental protocols were conducted according to guidelines of Ethics of Animal Use in Research Committee, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Isolation and Identification of *E. coli*

Rectal swabs were cultivated into nutrient broth (Difco) tubes and incubated aerobically at 37°C for 18 hours. A loopful from each broth culture was inoculated onto MacConkey agar (Oxoid) (Cruickshanak et al., 1975) and Eosin Methylene Blue (EMB) agar (Oxoid) (Koneman et al., 1988). Agar plates were incubated aerobically for 24 hours at 37°C and then were observed to detect if any bacterial colonies were present. Suspected colonies were assessed for Gram`s reaction. Gram-negative bacilli colonies were found. Each sample was further subjected to biochemical testing such as catalase, urease test, Triple Sugar Iron agar (TSI), methyl red test, indole test, citrate test, and Voges-Proskauer (IMViC) (McFaddin, 1985) as the traditional method of identification. Then, results were confirmed by VITEK2 compact identification and serotyping.

VITEK2 Compact System Identification

Identification by VITEK2 compact system was done according to the manufacture's instruction (Biomeriux VITEK-2 Compact ref Manual – Ref-414532- France) (Pincus, 2006).

Serological Identification

Ten *E. coli* isolates were submitted to slide agglutination test via polyvalent antisera per instruction of manufactures procedures (*E. coli* antisera Denka Seiken Co. LTD) (Collee et al., 1996).

Antimicrobial Susceptibility Testing

Fresh cultures of all isolates of *E. coli* were tested for antimicrobial susceptibility via the standard disk diffusion method as per guidelines of the Clinical Laboratory Standards Institute (CLSI, 2017). The following antimicrobial discs were used: amikacin (AK, 30 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), ampicillin (Amp, 15 µg), cephalothin (KF, 30 µg), ciprofloxacin (CIP, 30 µg), doxycycline (DO, 30 µg), erythromycin (E, 5µg), neomycin (N, 30 µg), tetracycline (TE, 30 µg), and trimethoprim/sulphamethoxazole (SXT, 25 µg) (Oxoid).

Detection of Some Virulence and Antibiotic Resistance Genes of *E. coli* by PCR

DNA was extracted from the seven *E. coli* serotypes to detect virulence and antibiotic resistance genes via the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's recommendations. 200 µl of the sample suspension was incubated with 200 µl of lysis buffer and 10 µl of proteinase K at 56°C for 10 minutes. Afterward, 200 µl of ethanol (100%) was added to the lysate. The sample was rinsed and centrifuged as per the instructions of the manufacturer. Nucleic acid was eluted with 100 µl of elution buffer found in the kit. The used primers sets were procured from Metabion (Germany). Table 1 illustrates the cycling parameters.

Primers were applied in a 25- µl reaction mixture of 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each 20 pmol primer concentrations, 6 µl of DNA template and 4.5 µl of water.

PCR outcomes were dispersed by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature utilizing gradients of 5V/cm. For gel examination, 20 µl of the PCR products were loaded in each gel slot. Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was adopted to know the fragment sizes. A gel documentation system (Alpha Innotech, Biomet

Table 1: Primers sequences, target genes, amplicon sizes, and cycling conditions of *E. coli*

Target gene	Primers Sequences	ze (bp)	Primary Denaturation	Amplification (35 cycles)			Final Extension	Reference
				Secondary Denaturation	Anneal-ing	Exten-sion		
eaeA	F: ATG CTT AGT GCT GGT TTA GG	248	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	72°C 7 min	Bisi-Johnson *et al.*, 2011
	R: GCC TTC ATC ATT TCG CTT TC							
stx1	F:ACACTGGATGATCT-CAGTGG	614						
	R:CTGAATCCCCCTC-CATTATG		94°C 5 min.	94°C 30 sec	58°C 40 sec	72°C 45 sec	72°C 10 min	Dipineto *et al.*, 2006
stx2	F:CCATGACAACGGA-CAGCAGTT	779						
	R:CCTGTCAACTGAG-CAGCACTTTG							
tetA	F:GGT-TCACTCGAACGACGT-CA	576	94°C 5 min	94°C 30 sec	50°C 40 sec	72°C 45 sec	72°C 10 min	Randall *et al.* 2004
	R:CTGTCCGACAA-GTTGCATGA							
blaTEM	F;ATCAGCAATAAAC-CAGC	516	94°C 5 min	94°C 30 sec	54°C 40 sec	72°C 45 sec	72°C 10 min	Colom *et al.*, 2003

ra) was used to get the gel photograph. Computer software was utilized to analyze the data.

RESULTS

Out of the 90 examined rectal swabs, 61 were positive for *E. coli* (67.7%) at different ages. The highest isolation rate was in puppies of less than three months old and recorded as 75.5% and the lowest frequency was 57.1% was recorded from dogs of more than one year old as shown in Table 2. Ten isolates were representing seven serogroups including O18, O27, O55, O126, O148, O158, and O166 in addition to 3 untypable groups.

Table 2: Occurrence of *E. coli* in diarrheic dogs of different ages,

Age of Dogs	No. of Examined Samples	No. of *E. coli* Positive samples	
		No.	%
≤ 3 months	53	40	75.5
> 3 months – 6 months	18	11	61.1
> 6 months–≤ 9 months	12	6	50.0
Over one year	7	4	57.1
Total	90	61	67.7

All isolates of *E. coli* subjected to disc diffusion sensitivity test showed high drug resistances levels (100%) was against tetracycline and trimethoprim/ sulphamethoxazole, followed by cephalothin, (95.1%) and erythromycin (93.4%), while the highest sensitivity (88.5%) was to amikacin (Table 3).

Table 3: Antimicrobial susceptibility of *E. coli* isolates (n=61)

Antibiotics	Resistant Isolates		Sensitive Isolates	
	%	No.	%	No.
Tetracycline (TE)	100	61	0	0
Trimethoprim/Sulphamethoxazole (SXT)	100	61	0	0
Cephalothin (KF)	95.1	58	4.9	3
Erythromycin (E)	93.4	57	6.6	4
Ciprofloxacin (CIP)	90.2	55	9.8	6
Doxycycline (DO)	83.6	51	16.4	10
Amoxicillin/Clavulanic Acid (AMC)	82	50	18	11
Ampicillin (Amp)	59	36	41	25
Neomycin (N)	32.8	20	67.2	41
Amikacin (AK)	11.5	7	88.5	54

The *E. coli* virulence genes of the tested isolates showed amplicons of 779, 614, and 248 bp for *stx*1, *stx2*, and *eae* genes, respectively. Of the seven-tested *E. coli* isolates (seven serogroups) five strains were positive to *stx1* gene, two strains to *stx2* gene, and four strains to *eae* gene (Table 4 and Figures 1 and 2). While the *bla TEM* and *tetA* antibio-

Table 4: Detection of virulence and multidrug resistance genes in *E. coli* serotypes isolated from diarrheic dogs.

Strain No.	Serogroup	Virulence Genes			Drug-Resistant Genes	
		eaeA	stx2	*stx1*	tetA	blaTEM
1	O27	+	+	-	+	+
2	O166	-	-	+	+	+
3	O126	-	-	+	+	+
4	O158	+	-	+	+	+
5	O148	+	-	+	+	+
6	O18	-	+	+	+	+
7	O55	+	-	-	+	+

(Absent); + (Present)

tic-resistant genes representing 516 and 576 bp size, respectively, were detected in all the seven tested isolates (seven serogroups) (Table 4 and Figures 3 and 4).

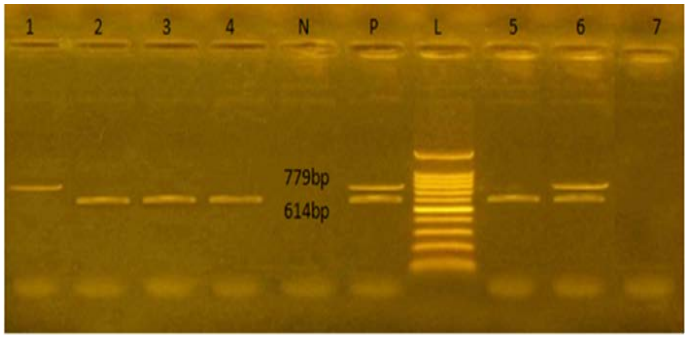

Figure 1: Agarose gel electrophoresis showed PCR products of *E.coli stx1*and stx2 genes amplified at 614 and 779 bp respectively from seven isolates. L: representing the molecular size marker (100 pb plus ladder). Lane 1 was positive to *stx2*, lanes 2,3,4, and 5 were positive to *stx1*. Lane 6 was positive to *stx1* and *stx2,* and lane 7 was negative to *stx1* and *stx2*. N: represents control negative, P: control positive

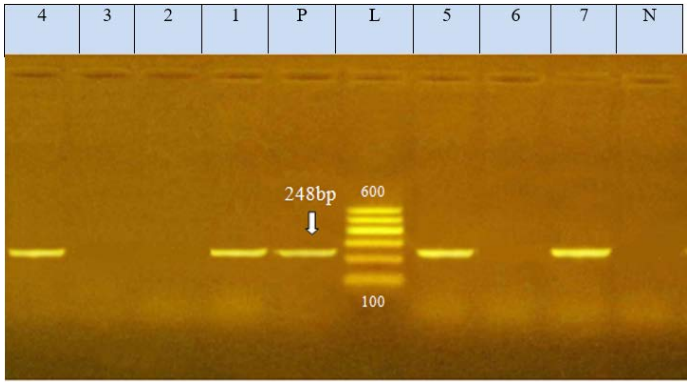

Figure 2: Agarose gel electrophoresis showed PCR product of *E.coli eae* gene amplified at 248 bp from seven isolates, N: represents negative control; P: represents positive control of *eae* gene (248 bp). L: represents the molecular size marker (100pb plus ladder). Lanes 1, 4, 5, and 7 were positive to *eae* gene. Lanes 2, 3, and 6 were negative to *eae* gene.

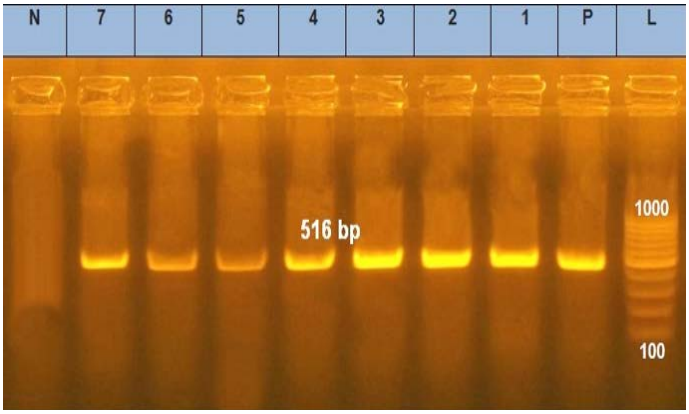

Figure 3: Agarose gel electrophoresis showed PCR products of *E .coli blaTEM.* Lane L was within 100–1000 bp DNA ladder. N: negative control; P: positive control of *blaTEM* gene 516 bp. Lanes 1–7 were positive to *bla TEM* gene

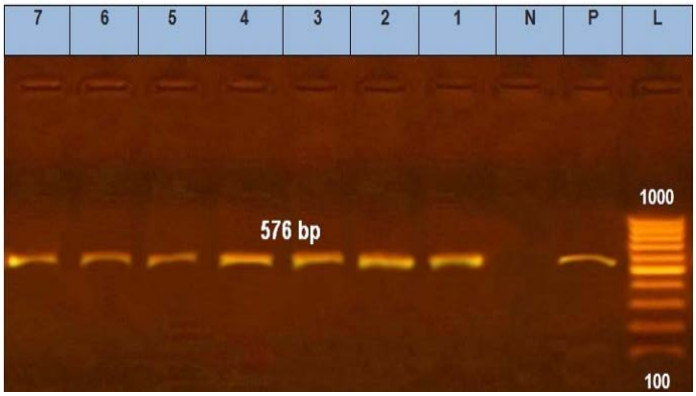

Figure 4: Agarose gel electrophoresis showed PCR products of *E. coli tetA*. N: represents negative control. P: represents positive control of *tetA* gene (576 bp). Lane L was within 100–1000 bp DNA ladder (1–7) positive to *tetA* gene.

DISCUSSION

Dogs and puppies are one of the critical carriers of *E. coli*, one of the chief causatives of diarrhea and other diseases in humans (Hasan et al., 2016). Out of the 90 examined

rectal swabs, 61 were positive for *E. coli* (67.7%) at different ages. The highest isolation rate was in puppies of less than three months old and recorded as 75.5% and the lowest frequency was 57.1% was recorded from dogs of more than one year old.

In this study, *E. coli* was isolated from diarrheic dogs at different ages, with total isolation percentages of 67.7% (61/90). Puppies under three months old were the most susceptible to *E. coli* infection; 75.5% of this pool agrees with Broes et al. (1988) who reported that *E. coli* was present in diarrheic puppies from one month to one month and a half old. Dogs older than one year were less susceptible to *E. coli* infection (Table 2). This conforms to various studies exhibiting that the incidence peak of enteritis always occurred within few days after the inception of the weaning period (one month) and the majority of EPEC infections take place in the first three months of lifespan as mentioned by Janke et al. (1989).

From all suspected isolates proved by traditional bacteriological examination to be *E. coli,* ten random isolates were reinvestigated using the VITEK2 compact system via Gram-negative cards and the results confirm the isolation of *E. coli* with an incidence of 100%. The VITEK2 compact system is a highly automated new tool for accurate and rapid identification of *E. coli*. The ten random isolates were subjected to serotyping and the results showed that these ten isolates were representing seven serogroups including O18, O27, O55, O126, O148, O158, and O166 in addition to 3 untypable groups.

On contrary, in other studies in Trinidad, Adesiyun et al. (1997) reported that the prevalence of EPEC strains was not significantly associated with age. Moreover, Marjanca et al. (2002) isolated 24 strains of hemolytic *E. coli* from dogs with diarrhea. The detected serogroup detected in this study were O18, O27, O55, O126, O148, O158, and O166. On the other hand, Maniam et al. (2020) reported that the most prevalent *E. coli* serogroup isolated from dogs from feces of healthy ones were O104:H4 and O102:H18. Also, Michele et al. (2014) found that *E. coli* O145: H 28 was isolated from stray dogs.

All isolates of *E. coli* subjected to disc diffusion sensitivity test showed high drug resistances levels (100%) was against tetracycline and trimethoprim/ sulphamethoxazole, followed by cephalothin, (95.1%) and erythromycin (93.4%), while the highest sensitivity (88.5%) was to amikacin only (Table 3).

Antimicrobial resistance is constantly evolving since the inception of antibiotics. Many aspects increase bacterial resistance such as disregarding treatment regimen, prophylactic consumption of antibiotics, and using antibiotics as growth promoters (Grave and Tanem, 2006). In our study, many strains showed multiple resistances to tetracycline, and trimethoprim/sulphamethoxazole (100%), cephalothin (95.1%), and erythromycin (93.4%), while the highest sensitivity (88.5%) was to amikacin. This agrees with the results obtained by Younis et al. (2015) that showed high resistance to trimethoprim/sulfamethoxazole (98%), erythromycin (97%), and cefotaxime (95%), while there was high sensitivity to amikacin (95%). Guardabassi et al. (2004) exhibited high sensitivity to amikacin. Habib et al. (2016) reported that *E. coli* was moderately resistant to tetracycline (54.33%), gentamycin (49.60%), Vibramycin (46.45%), ceftriaxone (44.88%), kanamycin (52.75%), ampicillin (59.65%), ciprofloxacin (25.98%) and norfloxacin (30.70%). These reports and the current study's findings indicate exponentially increasing resistance patterns of *E. coli*. It may also imply that *E. coli* in pet animals transmitted from human represent a G-directional public health hazard (Patoli et al., 2010).

The *E. coli* virulence genes of the tested isolates showed amplicons of 779, 614, and 248 bp for *stx*1, *stx2,* and *eae* genes, respectively. Of the seven-tested *E. coli* isolated, seven serogroups: five strains were positive to *stx1* gene, two strains to *stx2* gene, and four strains to *eae* gene. While the *bla TEM* and *tet A* antibiotic-resistant genes representing 516 and 576 bp size, respectively, were detected in all the seven tested isolates (seven serogroups).

In this study, virulence genes have been detected in *E.coli* isolated of dog origin where the *stx1* and *stx2* genes were detected in five *E. coli* serogroup (O166, O126, O158, O148, and O18) and two *E. coli* groups (O27 and O18), respectively. *stx* 1 and *stx* 2 genes maintain the same pattern in the other reports (Staats et al., 2003; Nakazato et al., 2004; Bentancor et al., 2007).

Herein, the *eaeA* gene was detected in four strains of different serogroups (O27, O185, O148, and O55). Similarly, Nakazato et al. (2004) reported the *eae*-positive isolates related to diverse serogroups, including O111:H25, O119:H2, and O142:H6. One *E. coli* (O18) strain in our work harbored both *stx1* and *stx2* genes. These results nearly agreed with the findings of Barkalita et al. (2016) who detected both stx1 and stx2 genes in O43 and O130 strains.

In our results, the *blaTEM* gene was detected in seven tested isolates by PCR at 516 bp, which agrees with the earlier findings (Younis et al., 2015; Delmani et al., 2017). The *blaTEM* gene found in a plasmid showed a strong correlation between the genogroup conferred resistance determined by PCR and antibiotic susceptibility patterns.

Moreover, *tetA* gene was found in seven tested isolates at 576 bp amplicon which agrees with (Nde and Logue, 2008) and (Torkan et al., 2015) who suggested that *tetA* could be the predominant tetracycline gene of resistance harbored by pathogenic *E. coli* present in dogs in Iran. There are other tetracycline-resistant genes thought to induce resistance via enzymatic inactivation and ribosomal protection.

CONCLUSION

Virulence genes of *E. coli* isolated from dogs were *stx1, stx2,* and *eae,* detected in current study, which probably influence the *E. coli* enteritis pathogenicity. Moreover, multidrug resistance genes were *blaTEM* and *Tet A* detected in *E. coli* isolates. The results suggested the pet owners to adopt hygiene practice to prevent infection, such as proper food-preparing methods, avoiding drinking water from potentially contaminated sources, thoroughly cooking meat before feeding dogs, and washing hands frequently and thoroughly.

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CONFLICT OF INTEREST

The authors declare that they no conflict of interest.

AUTHORS CONTRIBUTION

Z.Z and A.A planed the work. All authors conducted the practical part of the work and analysed the data. All authors contributed to the article and approved the submitted version.

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