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Research Article

Serotypes and Antibiotic Resistance of *Escherichia coli* Isolated from Canines and Felines

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Abstract | *Escherichia coli* (*E. coli*) are intestinal bacteria that affect people and animals like canines and felines. Pets are normally in close contact with their owners, and so harmful microorganisms can be easily transmitted from them to human beings. The ongoing review applied to be aware assuming canines and felines in Egypt are colonized with unsafe *E. coli* serotypes and the antibiotic resistance in these *E. coli* isolates. A total of 129 rectal swabs were gathered from apparent healthy and diarrheic canines and felines. By using Vitek2 compact system, 42 *E. coli* isolates (32.6%) were resulted from canines (24%) and felines (44.4%) rectal swabs. *E. coli* were serotyped to: O8, O25, O26, O28, O36, O55, O78, O86, O111, O114, O125, O127, O128 and O157. The resistance to 16 antibiotics and the creation of extended-spectrum β-lactamases (ESBLs) were identified on *E. coli* isolates by using Vitek2compact system. The creation of ESBL was distinguished in 5 of the isolated *E. coli*. The highest resistance was toward ampicillin (60%) and trimethoprim sulfamethoxazole (45%). No resistant was observed to piperacillin/tazobactam, meropenem & amikacin. The present study concluded that canines and felines carry diarrheic and multidrug resistant *E. coli* serotypes which have a public health concern. Attention should be paid to the contact with canines and felines, and the occurrence of multidrug resistance.

Keywords | Canines, Diarrhea, *E. coli* Serotypes, Felines, Multidrug resistance, Public health concern.

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INTRODUCTION

Escherichia coli are an intestinal bacteria that affects people and animals like chicken (El-Jakee et al., 2012; Desouky et al. 2021), ducks (Soliman et al., 2018), cattle (Kandil et al., 2011, El-Jakee et al., 2012, Daif et al., 2013), calves (Ismail et al., 1993), dogs (Banik et al., 2016), and cats (Rzewuska et al., 2015). *E. coli* which causes diarrhea are grouped: Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), and Diffuse-Adhering *E. coli* (DAEC) (Fallah et al., 2021). Shiga toxin-producing *E. coli* are a cause for serious infections worldwide, especially in children and elders. Its infection ranges from lower degree of diarrhea to hemorrhagic colitis, hemolytic uremic syndrome (HUS) and kidney failure, and in some cases, 2% death rate during the intense stage (Ibarra et al., 2013; Koochakzadeh et al., 2014; Mele et al., 2014; Galarce et al., 2020).*E. coli* O157 is the most often connected with cases of HUS in individuals (Vally et al., 2012), despite the fact that non-O157 STEC like O8, O25, O26, O28, O36, O103, O111, O121, and O145, have been related with serious illness (Gould et al., 2013), so the early identification

of STEC *E. coli* enables fast interference and control of the infection before it reaches HUS. The infection with STEC ranged from lower degree of diarrhea to severe forms of Hemorrhagic enteritis especially in puppies and kittens (Hasan et al., 2016; Yousif et al., 2016; Priya et al., 2017). STEC can be transmitted through the direct contact between individuals and pets besides, the contamination with its feces and urine. Consequently, presence of companion canines and felines in contact with human resembles a source of spread of health hazard diseases (Johnson et al., 2001; Bentancor et al., 2007).

Treatment of canines and felines with antibiotic agents decreases the shedding of STEC, so, decreases the spread of infection. The danger of STEC isn't only the diseases they cause, but also the multidrug resistance (MDR) they may spread. So, canines and felines must be treated with suitable antibiotic agents and with suitable doses. The worldwide spread of MDR *E. coli* was due to, no severe guidelines for the utilization of medications like β-lactams, fluoroquinolones, and sulfonamides. (Kennedy et al., 2017). Recently, the ownership of companion canines and felines has spread between teenagers and children, as well as people with disabilities, especially blind. But these animals are considered as a source of public health hazard. So, this study directed to be aware if canines and felines in Egypt are colonized with hurtful *E. coli* serotypes and then, detect their antimicrobial resistance through identifying and serotyping the isolated *Escherichia coli* using new trial of VITEK-2 compact method. Detection of the antimicrobial resistance (AMR) will help the control policies of using these compounds in animal husbandry, to assess the potential impact in the public health, and give updated data to national and international AMR surveillance programs.

MATERIALS AND METHODS

Samples

Rectal swabs were collected from 75 male and female canines (Husky, Griffon, Rottweiler, Golden, Half Golden, German shepherd, Labrador, Pit bull and Black coat), and 54 felines (Persian and Himalayan chocolate) aged from one month up to 2 years as shown in Table (1).

Under aseptic conditions, the swabs were gathered from apparently healthy and diarrheic Canines and Felines, and then transported to the laboratory in ice box for further bacterial examination. The samples were collected from animals according to ethical guidelines of the Institutional Animal Care & Use Committee (IACUC) at the Faculty of Veterinary Medicine and Cairo University.

Cultivation and isolation of *E. coli*

Rectal swabs were incubated at 37 ˚C for 24 h after cultivation on MacConkey agar and Eosin Methylene Blue agar (EMB) plates (Difco, USA). The suspected colonies were gathered for morphological and biochemical characterization by traditional methods as previously described by MacFaddin (Schau, 1986).

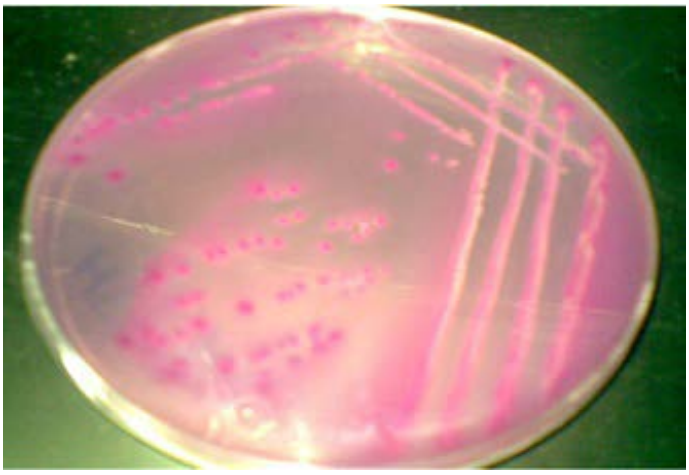

Figure 1: MacConkey agar

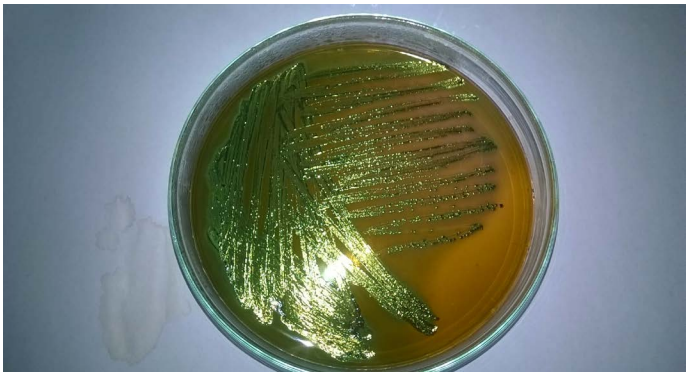

Figure 2: Eosin Methylene Blue agar

Identification of *E. coli* isolates by Vitek2 compact system

All characteristic isolates were identified by Vitek2 compact system and special ID-GN for identification of gram negative bacteria according to manufacture structure (BioMerieux, 2006).

Serogrouping of the isolates

Characterized *E. coli* isolates were serotyped by utilization of specific *E. coli* antisera (Sifin diagnostics gmbh, Berlin, Germany) (Starr, 1986).

The antimicrobial susceptibility testing (AST) of the isolates

Antibiotic resistant test was carried out using Vitek2 compact system and special AST-GN73 cards for antimicrobial susceptibility test of Gram`s negative bacteria, according to manufacture structure (BioMerieux, 2006).

Table 1: Samples collected from dogs and cats of different ages, sex and breeds

Breed	Age	Sex	Number	Breed	Age	Sex	Number
Dogs				Cats			
Husky	1-3 months	M	4	Persian	1-3 months	F	7
	3-9 months		2		3-9 months		3
Griffon	1-3 months	F	3		> 9 months		5
	3-9 months		3		1-3 months	M	15
	1-3 months	M	5		3-9 months		9
	3-9 months		4		> 9 months		12
Rottweiler	1-3 months	F	3	Himalayan chocolate	1-3 months	F	3
	3-9 months	M	3				
Half Golden	1-3 months	M	3				
Golden	1-3 months	M	3				
German shepherd	1-3 months	F	15				
	1-3 months	M	10				
	9 months	M	8				
Labrador	3-9 months	M	3				
Pit bull	1-3 months	F	3				
Black coat	3-9 months	F	3				
Total samples	75			54			

M: male, F: female

Table 2: Prevalence of the isolated *E. coli* from dogs and cats.

Source	Apparently healthy			Diarrheic			Total		
	Number of examined animals	Positive	%	Number of examined animals	Positive	%	Number of examined animals	Positive	%
Dogs	25	5	20.0	50	13	26.0	75	18	24.0
Cats	18	7	38.9	36	17	47.2	54	24	44.4
Total	43	12	27.9	86	30	34.9	129	42	32.6

Table 3: Prevalence of the *E. coli* from dogs and cats according to their age.

Age	Dogs				Cats			
	Male		Female		Male		Female	
	No	PNo/ %	No	PNo/ %	No	PNo/ %	No	PNo/ %
1-3 month	25	33.3	24	32	15	27.7	10	18.5
3-9 months	12	16.0	6	8	9	16.6	3	5.5
Above 9 m0nths	8	10.6	0	0	12	22.2	5	9.2
Total	45	60.0	30	40	36	66.6	18	33.3

PNo= positive number of *E. coli*

Table 4: Prevalence of *E. coli* serotypes collected from dogs and cats

Serotyping	Dogs						Cats						Total (129)	
	Apparently healthy (25)		Diarrheic (50)		Total (75)		Apparently healthy (18)		Diarrheic (36)		Total (54)			
	No	%	No	%	No	%	No	%	No	%	No	%	NO	%
O8	0	0	1	2.0	1	1.3	0	0	1	2.8	1	1.9	2	1.6

Links Researchers

O 25	1	4.0	1	2.0	2	2.7	1	5.6	1	2.8	2	3.7	4	3.1
O 26	0	0	1	2.0	1	1.3	0	0	1	2.8	1	1.9	2	1.6
O 28	0	0	1	2.0	1	1.3	0	0	1	2.8	1	1.9	2	1.6
O 36	0	0	0	0	0	0	0	0	2	5.6	2	3.7	2	1.6
O 55	0	0	1	2.0	1	1.3	0	0	1	2.8	1	1.9	2	1.6
O 78	1	4.0	1	2.0	2	2.7	0	0	0	0	0	0	2	1.6
O 86	1	4.0	1	2.0	2	2.7	1	5.6	1	2.8	2	3.7	4	3.1
O 111	0	0	0	0	0	0	0	0	1	2.8	1	1.9	1	0.8
O 114	0	0	1	2.0	1	1.3	0	0	1	0	1	1.9	2	1.6
O 125	1	4.0	3	6.0	4	5.3	2	11.1	2	5.6	4	7.4	8	6.2
O 127	0	0	0	0	0	0	1	5.6	1	2.8	2	3.7	2	1.6
O 128	1	4.0	0	0	1	1.3	1	5.6	2	5.6	3	5.6	4	3.1
O 157	0	0	1	2.0	1	1.3	0	0	1	2.8	1	1.9	2	1.6
Untypable	0	0	1	2.0	1	1.3	1	5.6	1	2.8	2	3.7	3	2.3
Total	5	20.0	13	26.0	18	24.0	7	38.9	17	47.2	24	44.4	42	32.6

No = positive number

% was calculated according to number of the examined animals

Table 5: Antibiotic resistance pattern of *E. coli* isolates.

Antibacterial agents	Sensitive		Intermediate		Resistance	
	No	%	No	%	No	%
Ampicillin	8	40.0	0	0	12	60.0
Ampicillin/sulbactam	13	65.0	0	0	7	35.0
Pipercillin/Tazobactam	20	100.0	0	0	0	0
Cefazolin	13	65.0	0	0	7	35.0
Cefoxitin	17	85.0	0	0	3	15.0
Ceftazidime	15	75.0	0	0	5	25.0
Ceftriaxone	15	75.0	0	0	5	25.0
Cefepime	16	80.0	0	0	4	20.0
Meropenem	20	100.0	0	0	0	0
Amikacin	20	100.0	0	0	0	0
Gentamicin	14	70.0	3	15.0	3	15.0
Tobramycin	18	90.0	0	0	2	10.0
Ciprofloxacin	16	80.0	2	10.0	2	10.0
Levofloxacin	15	75.0	0	0	5	25.0
Nitrofurantoin	18	90.0	2	10.0	0	0
Trimethoprim/Sulfamethoxazole	11	55.0	0	0	9	45.0

RESULTS

The phenotypic characterization of *E. coli*

Forty two *E. coli* isolates from rectal swab samples of 129 canines and felines with the percentage (32.6%) as shown in Table (2) produced characteristic pink color on MacConkey agar as shown in Figure (1) and metallic sheen color on EMB agar as shown in Figure (2). All the isolates were identified biochemically by GN card of Vitek 2 compact system (bioMe´rieux), and all isolates were confirmed as *E. coli*. *E. coli* were isolated from canines and felines rectal swabs with prevalence 24% (18/75) and 44.4% (24/54) respectively as shown in Table (2). Most of *E. coli* isolates were collected from diarrheic animals (34.9%) than apparently healthy animals (27.9%). In canines, the incidence of *E. coli* was higher in males (60%) than in females (40%). In felines, the incidence of *E. coli* isolated from males (66.6%) was higher than that isolated from females (33.3%). Besides, most *E. coli* was isolated from puppies and kittens (1-3 months) as shown in Table (3).

Serogrouping of *Escherichia coli*

Out of the 42 isolated *E. coli* strains, 39 isolates (92.8%) were shown to belong to 14 O serogroups: O8, O25, O26, O28, O36, O55, O78, O86, O111, O114, O125, O127, O128 and O157. Furthermore, three isolates (7%) were untypable as shown in Table 4. In canines, O8, O25, O78, O86 and O125 were obtained from diarrheic and apparently healthy pets. While, in felines, O25, O86, O125, O127, O128, and untypable were collected from diarrheic and apparently healthy pets.

Antibiotic resistance of *E. coli*

The creation of ESBL was distinguished in 5 *E. coli* isolates. The most common resistance was recorded against ampicillin (60%), trimethoprim/sulfamethoxazole (45%), ampicillin/sulbactam (35%) and cefazolin (35%). The lower resistance was toward gentamicin (15%), ciprofloxacin (10%) and nitrofurantoin (10%). The resistance to levofloxacin, ceftriaxone and ceftazidime was 25% each. Some of *E. coli* isolates gave resistance to cefepime (20%), cefoxitin (15%) and tobramycin (10%). No resistant was detected against piperacillin/tazobactam, meropenem and amikacin as shown in Table (5).

DISCUSSION

The current review was carried out to examine incidence, serotypes and antibiotic resistance of *E. coli* isolated from apparently healthy and diarrheic canines & felines. The clinically examined diarrheic canines and felines showed different signs of illness like: fever, elevated respiratory rate and heart rate, yellowish to bloody diarrhea and dehydration. These outcomes agreed with those reviewed by Yousif et al. (2016). Additionally, the phenotypic characters of the disengaged *E. coli* like got by Sengupta et al. (2011). The incidence rate of *E. coli* isolated from canines is very much like that articulated in France (24.5%) by Haenni et al. (2014). Besides, a lower rate was articulated in Tunisia (17.5%) by Sallem et al.(2013) and a higher rate of *E. coli* was articulated in Egypt (37.14%) by Ali and Metwally (2015). Also the rate of *E. coli* in cats is intently like that got by a past report in Poland (45.1%) by Rzewuska et al. (2015) and higher than the incidence reviewed in Brazil (2.5%) by Puño-Sarmiento et al. (2013). In Egypt Younis et al. (2015) recorded a higher incidence of *E. coli* (67%). Infection with *E. coli* is higher in male than females, which was inconsistent with Tahamtan et al. (2011) who observed that infection with *E. coli* is higher in female than males. *E. coli* isolation was higher in younger ages compared to older ones and higher in diarrheic than non- diarrheic, this means that healthy pets can harbor *E. coli* without any signs of illness and act as a carrier. This agrees with that obtained by Coura et al. (2018). This diversity is mainly due to the geographical variance, type of food, differences in the health status, and the hour of examinations (Carvalho et al., 2021). Considering *E. coli* serotyping, the serotypes, O8, O25, O26, O28, O36, O55, O78, O86, O111, O114, O125, O127, O128 and O157 was closely similar to those resulted by Ali and Metwally (2015); Banik et al. (2016), and Algammal et al. (2022). The investigated serotypes reflect the epidemiological and general wellbeing significance.

In the existing work, *E. coli* afforded an antibiotic resistance toward ampicillin, trimethoprim sulfamethoxazole, cefazolin, levofloxacin, ceftriaxone, ceftazidime, cefepime, gentamicin, cefoxitin, ciprofloxacin and tobramycin. Additionally, some of these isolates harbor extended- spectrum β-lactam (ESBL) which is an incredibly undermining public health. These records agree with those got by Torkan et al. (2016); Wedley et al. (2017) and Algammal et al. (2022).Recently, the antimicrobial resistance has extended worldwide due tothe uncontrolled application of the antimicrobial agents in the health and veterinary sectors. Although antimicrobials are commonly used for treatment of diseases, infections caused by antimicrobial resistant *E. coli* transferred from animals to humans could be even more difficult to be treated. Some strict measures are needed to limit the prevalence of MDR *E. coli* in animal reservoirs, consequently, reducing the use of antimicrobials as much as possible.

CONCLUSION AND RECOMMENDATIONS

Canines and Felines faeces can be a source of zoonotic diseases that presenting a threat to humans through their virulence factors or MDR. The non-hygienic maintaining of Canines and Felines may maximize the risk of colonisation of such pathogens in humans. So recommendations for appropriate use of antimicrobials in Canines and Felines treatment should be followed to decrease the occurance of multidrug resistance among *E. coli* in those animals which have clinical relevance and public health importance.

ETHICS APPROVAL

The samples were collected from animals according to ethical guidelines of the Institutional Animal Care & Use Committee (IACUC) at the Faculty of Veterinary Medicine and Cairo University.

NOVELTY STATEMENT

Here we present a Vitek2 compact system method to rapidly and accurately detect E. coli from pets as well as detect multidrug resistant isolates.

Two Shiga-like toxin–producing types of *Escherichia coli* O157 strains were isolated from dog and cat that can cause severe disease in humans and animals and may be a serious hazard to work with.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTION

Eman H. Abotalp and Sahar R. Mohamed verified the analytical methods. Jakeen K. El Jakee supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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