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ANTIBIOGRAM AND VIRULOTYPING PICTURE OF ENTERO-PATHOGENIC *ESCHERICHIA COLI* AND *KLEBSILA PNEUMONIAE* ISOLATED FROM DIARRHEIC CATS AND DOGS

By

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ABSTRACT

Nowadays, infectious diarrhea in dogs and cats is a frequent disease problem with pet animal veterinarians. The present study discussed the occurrence of some entero-pathogenic pathogens such as *E.coli*, *Klebseilla* in fecal samples of dogs and cats with the detection of virulence genes fecal samples were collected from 180 diarrheic dogs and 70 diarrheic were submitted to the 6th of October Veterinary.Enteropathogenic *E.coli* isolates were recovered from 42% (n=75/180) and 40% (n=28/70) of dog and cat samples, respectively. On the other hand,

K. pneumoniae incidence in dogs was 21% (n=37/180) and 13% (n=9/70) in cats. Antibiogram of the examined isolates revealed that 68% of dog isolates and 79% of cat isolates of *E. coli* highly resistant to ampicillin/sulbactam. The resistance to ampicillin and augmentin was conferred by (59%,64%) and (49%54%) of dog and cat isolates, respectively. Meanwhile,

K. Pneumoniae isolates from dogs and cats were highly resistant to ceftazidime (100%,100%), ampicillin (78% and 88%), amikin (78% and 67%), augmentin (62% and 67%) respectively. The molecular characterization of the isolates proved high frequency of the virulence genes *VT2e, eaeA, LT, STa, Cnf1, mrkA, ecpA, fimA* and *fimH*. In conclusion, it looks like that, the virulence genes detected in the isolates were linked with diarrhea in both types of tested pets at various ages. Potential active transmission of such types of bacteria to humans is expected; thus it represents an escalating potential public health.

Keywords:

E. coli ,Dog ,Cat , Antibiogram ,Virulence.

INTRODUCTION

The gastrointestinal tract of animals is colonized by an intensive and varied set of micro-organisms recognized as the GI microbiota that supplies more than 9,000,000 unique genes to the gene repertoires in the eukaryotic host **(Auchtung *et al.*, 2018).**

Many entero-pathogens are diagnosed in dogs and cats' feces, however they do not all have the possibility for human transmissions **(Demirbilek ,2017).** Dogs and cats have a significant function in modern societies, endorsing the psychological and physiological happiness of many individuals but there are well-known health dangers connected to human-animal interaction **(Kantere *et al.*, 2014).** The transmittance of pathogenic bacteria from animal to human being is increasing due to the relative relation between companion animals and humans in the new society. Such fact leads to a greater way of interspecies transmission of bacteria by different ways including fecal contamination **(Pomba *et al.*, 2017).** *E. coli* strains are part of the normal biota of human being and animals; however, several clinical records have blamed *E. coli* as the causative agent of diarrhea in human being and companion animals. **(Vega-Manriquez1 *et al.*, 2020).** In domestic dogs diarrhea is related to various bacteria, the utmost significant is enetropathogenic *E. coil* (EPEC) and enterotoxigenic *E. coli* (ETEC) strains **(Puño-Sarmiento *et al.*, 2013 and Adorján *et al.*, 2021).** EPEC strains have been detected in human patients and dogs living in the same house **(Rodrigues *et al.*, 2004).** The detaching of *E. coli* by friend animals in their feces represents a significant resource of zoonotic transmissions of infective agents **(Puño-Sarmiento *et al.*, 2013).** Companion dogs are carriers of infective bacteria and could spread them to their owners and individuals without direct contact with them **(Adorján *et al.*, 2021).** Moreover,*Enterobacteriaceae* types that result in urinary tract infection (UTI), like *K. pneumoniae* are a significant part of the host bowel microbiota **(Martin *et al.*, 2016).** *K. pneumoniae* is a grave nosocomial microorganism that is recognized to be disseminated simply **(Martin *et al.*, 2018).** *K. pneumoniae* lead to in infections in companion pets and people and is the second commonest *Enterobacteriaceae* class resulting in UTI in people **(Marques *et al.*, 2018).** The incidence of multidrug resistant bacteria is accompanying with the increased usage of wide-spectrumanti-microbials in the treatment ofhuman-animal infections**(Garcia-**

Mazcorro, 2013). Hence, the current study was managed to carry out molecular characterization of entero-pathogenic *E. coli* and *Klebsiella spp.* from diarrheic cats and dogs with a special focus on multi-drug resisting criteria.

MATERIAL AND METHODS

The current work comprised dogs and cats at different ages with complete records (Age, sex, breed, and existence or not of clinical symptoms) were submitted from private veterinary clinics at Cairo and Giza in Egypt. The target cases (180 dogs and 70 cats) were diarrheic ones. Fresh fecal samples were gathered from such animals, transported immediately cooled to the laboratory and subjected to bacteriological examination.

Isolation and biochemical characterization of the bacterial isolates:

Fecal samples were inoculated onto MacConkey's agar plates and incubated aerobically at 37°C for one day. After incubation, the plates were examined for the presence of bacterial colonies. Collected Colonies from each sample were subjected to Gram staining and bio-chemical test; urease including indole, methyl red, Voges-Proskauer and citrate utilization test in addition to reactions in triple sugar iron agar slopes according to methods described by **Marks *et al.* (2011).**

Antimicrobial Susceptibility testing:

The antibiogram disk diffusion technique was adapted according to Clinical and Laboratory **Standards Institute (2018)** through using antimicrobial disc of ampicillin,ciprofloxacin, imipenem,trimethoprim,doxycycline,azetronam,amikacin,gentamycin,ceftriaxone,ceftazidime , emoxy / clav, ampicillin/ sulbactam, and cefepime.

PCR detection of virulence genes:

Bacterial DNA was extracted using the QIA amp DNA mini kit (Qiagen, UK) and the manufactrer instructions were followed by virulence genes detection of the virulence genes in EPEC isolates was carried out by PCR using specific primers targeting certain sequences in the following genes; *pho*A **(Hu *et al.*, 2011),** *VT2e* **(Orlandi *et al.*, 2006),** *eae*A **(Bisi-Johnson *et al.*, 2011),** *LT* **(Lee *et al.*, 2008),** *STa* **(Lee *et al.*, 2008),** *Cnf1* **(Kadhum *et al.*, 2008),***Omp*A **(Ewers *et al.*, 2007).** Concerning Klebsiella isolates *gyr*A **(Brisse and Verhoef, 2001),** *mrkA-ecpA-fimA* **(Alcántar-Curiel *et al.*, 2018)** and *fimH* **(Ghanbarpour and Salehi, 2010) genes were targeted** (Table 5).

RESULTS

E. coli was isolated from 75 out of 180 dog fecal samples (42%) and 28 of 70 cats fecal samples (40%). On the other hand, *K. pneumoniae* was isolated from 37 out of 180 dog samples (21%) and 9 out of 70 cat samples (13%). The results of antimicrobial sensitivity test on *E.coli* (n=103) and *K. pneumoniae* (n=46) isolates are depicted in (Tables 1, 2, 3 and4). PCR screening of the isolates for the virulence genes indicated that *eaeA-LT- STa* and *OmpA* genes are harbpored by all tested *E.coli* isolates, while *Cnf1* and *VT2e* genes were not detected. In contrast, all *K. pneumoniae* tested isolates harbored the *mrk*A, *ecp*A, *fim*A and*fim*H genes. (Table 6), Fig. (1-5).

Table (1): Antibacterial sensitivity test of 75 *E. coli* isolates from diarrheic dog fecal samples.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	n =	%	n =	%	n =	%
Ampicillin	**0**	**0**	**31**	**41**	**44**	**59**
Amikacin	**54**	**72**	**8**	**11**	**13**	**17**
Emox/clav	**12**	**16**	**26**	**35**	**37**	**49**
Aztreonam	**75**	**100**	**0**	**0**	**0**	**0**
Ciprofloxacin	**75**	**100**	**0**	**0**	**0**	**0**
Ceftazidime	**24**	**32**	**15**	**20**	**36**	**48**
Ceftriaxone	**0**	**0**	**39**	**52**	**36**	**48**
Doxycycline	**40**	**53**	**30**	**40**	**5**	**7**
Imipenem	**67**	**89**	**0**	**0**	**8**	**11**
Gentamycin	**8**	**11**	**54**	**72**	**13**	**17**
Cefepime	**53**	**71**	**0**	**0**	**22**	**29**
Trimethoprime	**68**	**91**	**0**	**0**	**7**	**9**
Ampicillin/Sulbactam	**17**	**23**	**7**	**9**	**51**	**68**

Table (2): Antibacterial sensitivity test of 28 *E. coli* isolates from diarrheic cat fecal samples.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	n=	%	n=	%	n=	%
Ampicillin	**0**	**0**	**10**	**36**	**18**	**64**
Amikacin	**19**	**68**	**3**	**11**	**6**	**21**
Emox/clav	**4**	**14**	**9**	**32**	**15**	**54**
Aztreonam	**27**	**96**	**1**	**4**	**0**	**0**
Ciprofloxacin	**28**	**100**	**0**	**0**	**0**	**0**
Ceftazidime	**9**	**32**	**6**	**22**	**13**	**46**
Ceftriaxone	**0**	**0**	**15**	**54**	**13**	**46**
Doxycycline	**16**	**57**	**10**	**36**	**2**	**7**

Imipenem	**23**	**82**	**0**	**0**	**5**	**18**
Gentamycin	**2**	**7**	**21**	**75**	**5**	**18**
Cefepime	**20**	**71**	**0**	**0**	**8**	**29**
Trimethoprime	**27**	**96**	**0**	**0**	**1**	**4**
Ampicillin/Sulbactam	**4**	**14**	**2**	**7**	**22**	**79**

Table (3): Antibacterial sensitivity test of 37 *K. pneumoniae* isolates from diarrheic dog fecal samples**.**

Antimicrobial agent	**Sensitive**		**Intermediate**		**Resistant**	
	n=	**%**	**n=**	**%**	**n=**	**%**
Ampicillin	**4**	**11**	**4**	**11**	**29**	**78**
Amikacin	**4**	**11**	**4**	**11**	**29**	**78**
Emox/clav	**14**	**38**	**0**	**0**	**23**	**62**
Aztreonam	**21**	**57**	**4**	**11**	**12**	**32**
Ciprofloxacin	**37**	**100**	**0**	**0**	**0**	**0**
Ceftazidime	**0**	**0**	**0**	**0**	**37**	**100**
Ceftriaxone	**0**	**0**	**15**	**41**	**22**	**59**
Doxycycline	**22**	**59**	**11**	**30**	**4**	**11**
Imipenem	**37**	**100**	**0**	**0**	**0**	**0**
Gentamycin	**0**	**0**	**37**	**100**	**0**	**0**
Cefepime	**17**	**46**	**8**	**22**	**12**	**32**
Trimethoprime	**26**	**70**	**0**	**0**	**11**	**30**
Ampicillin/Sulbactam	**13**	**35**	**4**	**11**	**20**	**54**

Table (4): Results of antibacterial sensitivity test of 9 *K. pneumoniae* isolates from diarrheic cats fecal samples**.**

Antimicrobial agent	**Sensitive**		**Intermediate**		**Resistant**	
	n=	**%**	**n=**	**%**	**n=**	**%**
Ampicillin	**0**	**0**	**1**	**11**	**8**	**88**
Amikacin	**1**	**11**	**2**	**22**	**6**	**67**
Amoxy/clavulinic	**3**	**33**	**0**	**0**	**6**	**67**
Aztreonam	**5**	**56**	**1**	**11**	**3**	**33**
Ciprofloxacin	**9**	**100**	**0**	**0**	**0**	**0**
Ceftazidime	**0**	**0**	**0**	**0**	**9**	**100**
Ceftriaxone	**0**	**0**	**4**	**44**	**5**	**56**
Doxycycline	**5**	**56**	**3**	**33**	**1**	**11**
Imipenem	**9**	**100**	**0**	**0**	**0**	**0**
Gentamycin	**0**	**0**	**9**	**100**	**0**	**0**

Cefepime	**4**	**44**	**2**	**23**	**3**	**33**

Genes	Dogs			Cat		
	Results	n=isolates	%	Results	n=isolates	%
***E.coli* genes**						
VT2e	-	75/75	100	-	28/28	100
eaeA	+	75/75	100	+	28/28	100
LT	+	75/75	100	+	28/28	100
STa	+	75/75	100	+	28/28	100
Cnf1	-	75/75	100	-	28/28	100
ompA	+	75/75	100	+	28/28	100
mrkA	+	37/37	100	+	9/9	100
Klebsiella genes						
ecpA	+	37/37	100	+	9/9	100
fimA	+	37/37	100	+	9/9	100
fimH	+	37/37	100	+	9/9	100

Trimethoprime	**7**	**78**	**0**	**0**	**2**	**22**
Ampicillin/Sulbectam	**3**	**33**	**1**	**11**	**5**	**56**

Table (5): Primers used for *E. coli* and *K. pneumoniae* isolates from cat and dog fecal. samples.

Table (6): Genes detected in *E. coli* and *K. pneumoniae* isolated from dogs and cats samples.

Target agent	Gene	Sequence (5'-3')	Product	Reference
Klebsiella	***gyrA***	**CGC GTA CTA TAC GCC ATG AAC GTA**	**441 bp**	**Brisse and Verhoef, 2001**
		ACC GTT GAT CAC TTC GGT CAG G		
E. coli	***phoA***	**CGATTCTGGAAATGGCAAAAG**	**720 bp**	**Hu *et al.*, 2011**
		CGTGATCAGCGGTGACTATGAC		
	FimA	**CGGACGGTACGCTGTATTTT**	**436 bp**	**Alcántar-Curiel *et al.*, 2018**
		GCTTCGGCGTTGTCTTTATC		
	mrkA	**CGGTAAAGTTACCGACGTATCTTGTACTG**	**475 bp**	
		GCTGTTAACCACACCGGTGGTAAC		
	ecpA	**GCAACAGCCAAAAAAGACACC**	**477 bp**	
		CCAGGTCGCGTCGAACTG		
	fimH	**TGCAGAACGGATAAGCCGTGG**	**508 bp**	**Ghanbarpour and Salehi, 2010**
		GCAGTCACCTGCCCTCCGGTA		
E. coli	***STa***	**GAAACAACATGACGGGAGGT**	**229 bp**	**Lee *et al.*, 2008**
		GCACAGGCAGGATTACAACA		
	LT	**GGTTTCTGCGTTAGGTGGAA**	**605 bp**	
		GGGACTTCGACCTGAAATGT		

	eaeA	**ATG CTT AGT GCT GGT TTA GG**	**248 bp**	**Bisi-Johnson *et al.*, 2011**
		GCC TTC ATC ATT TCG CTT TC		
	Cnf1	**TATATAGTCGTCAAGATGGA**	**620 bp**	**Kadhum *et al.*, 2008**
		CACTAAGCTTTACAATATTGAC		
	Vt2e	**CCAGAATGTCAGATAACTGGCGAC**	**322 bp**	**Orlandi *et al.*, 2006**
		GCTGAGCACTTTGTAACAATGGCTG		
	ompA	**AGCTATCGCGATTGCAGTG**	**919 bp**	**Ewers *et al.*, 2007**
		GGTGTTGCCAGTAACCGG		

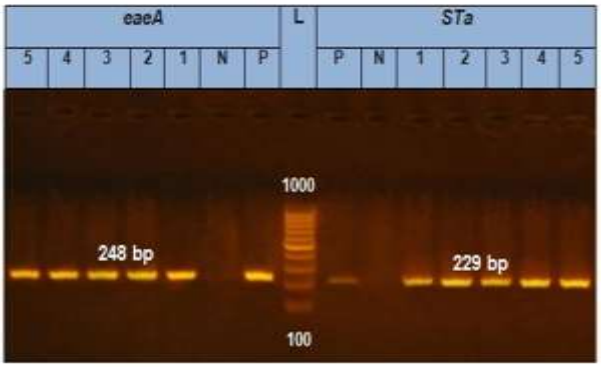

Fig (1): Agarose gel electrophoresis for PCR on *E. coli* isolates DNA for detection of *eae*A and *STa* genes at 248 bp. and 229 bp. respectively. Lane L: 100 bp DNA size marker, Lane P: control positive DNA. Lanes 1-5 right PCR products of the *st*a genes and lanes 1-5 left: PCR products of *eae*A gene.

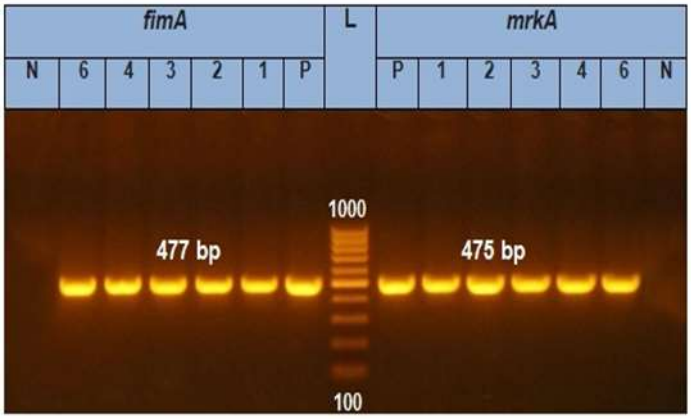

Fig (2): Agarose gel electrophoresis for PCR on *K. pneumoniae* isolates DNA for detection of *fim*A and *mrk*A genes at 477 bp. and 475 bp. respectively. Lane L:100 bp DNA size marker, Lane P: control positive DNA. Lanes 1-6 PCR products.

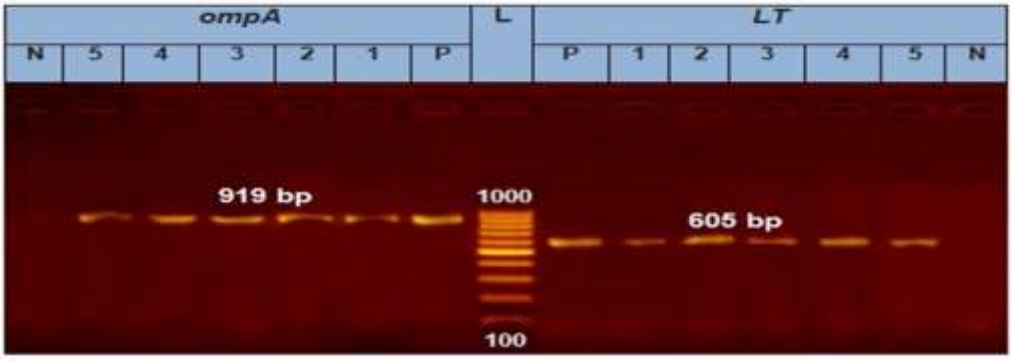

Fig (3): Agarose gel electrophoresis for PCR on *E. coli* isolates DNA for detection of *ompA* and *LT* genes at 919 bp. and 605 bp. respectively. Lane L: 100 bp DNA size marker, Lane P: control positive DNA. Lanes 1-5: PCR products.

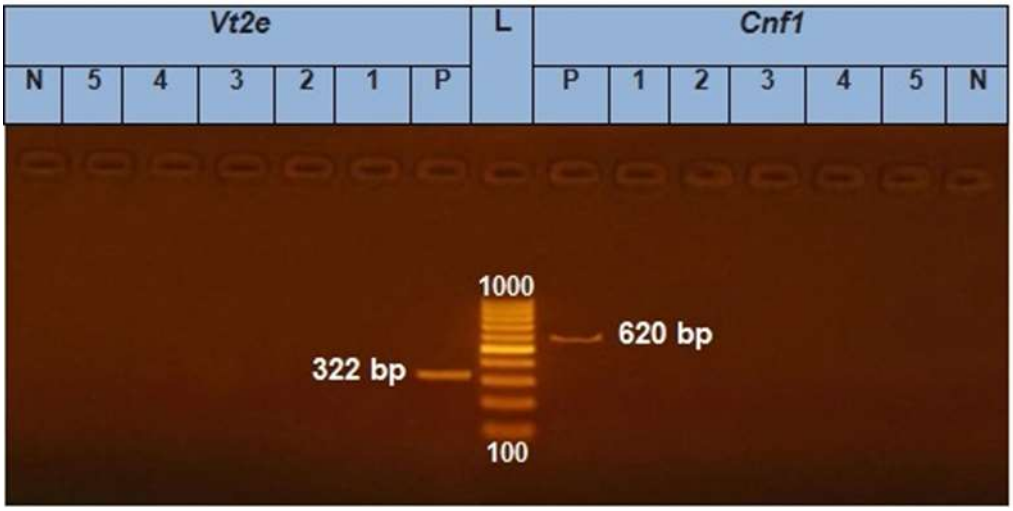

Fig (4): Agarose gel electrophoresis for PCR on *E. coli* isolates DNA for detection of *Vt2e* and *Cnf1* genes at 322 bp. and 620 bp. respectively. Lane L: 100 bp DNA size marker, Lane P: control positive DNA. Lanes 1-2: PCR products.

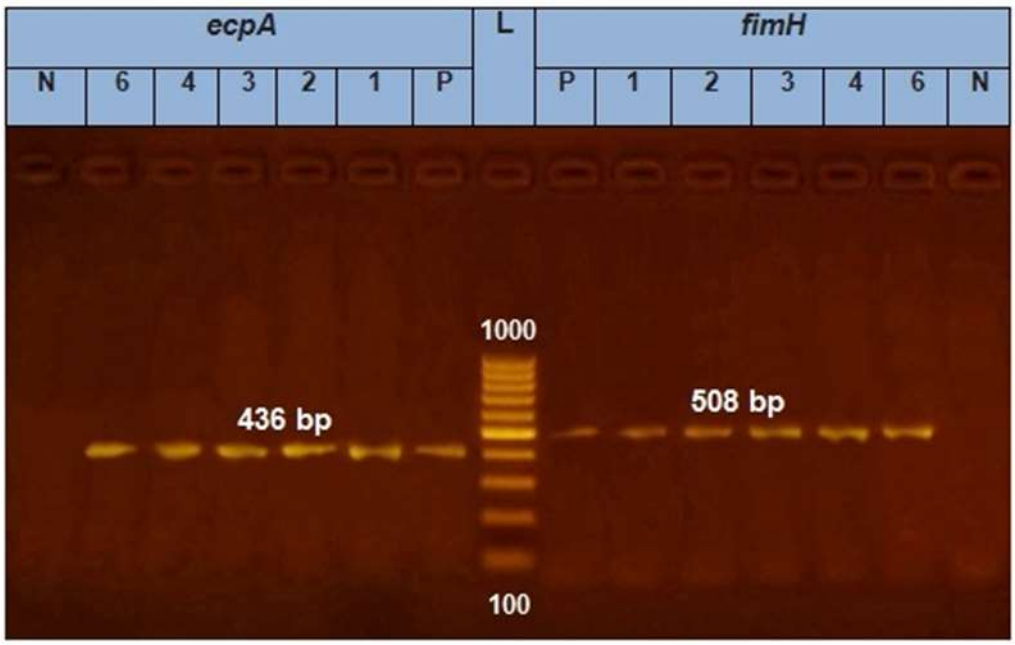

Fig (5): Agarose gel electrophoresis for *K. pneumoniae* isolates DNA for detection of *ecpA* and *fimH* genes at 436 bp. and 508 bp. respectively. Lane L: 100 bp DNA size marker, Lane P: control positive DNA.

(Positive and negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production,Animal health research institute) .

DISCUSSION

Escherichia coli is Gram-negative rod belonging to the family Enterobacteriaceae is a part of the normal flora of the gastrointestinal tract. However, *E. coli* can cause enterocolitis in the presence of bacterial virulence factors and impaired local or systemic immunity. Also some strains might be zoonotic. There are more than 170 serogroups of *E. coli* based on the identity of the bacterial O (Somatic) antigen, the external lipopolysaccharide-ride (LPS) and the H (Flagellar) antigens. *E. coli* strains that cause gastrointestinal diseases have been divided into seven clear pathogenic types, defined by a characteristic set of virulence factors responsible for the clinical, pathologic and epidemiologic features of the disease.

The seven types include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), necrotoxigenic *E. coli* (NTEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and adherent-invasive *E. coli* (AIEC) **(Bien *et al.*, 2011; Puno-Sarmiento *et al.*, 2013).** *E. coli* has been involved in several clinical cases of

diarrhea in dogs **(Beutin, 1999; Puno-Sarmiento *et al.*, 2013)**. All of the present study samples from dogs and cat were screened by conventional polymerase chain reaction (PCR) for pathotyping and for detection of virulence capability. The *eae*A gene was found in all *E. coli* isolates from dogs and cats in the present study, a findung that agrees with what was mentioned by **Jerse *et al.* (1990),** whom detected *eae*A gene in dogs and cats *E. coli* isolates. The existence of pathogenic *E. coli* strains in diarrhoeic companion animals is of major significance because of the high possibility of zoonotic transmission **(Geser *et al.*, 2011; Nguyen and Speradio, 2012).** These findings generally show that pets could harbor the entero-pathogenic (EPEC) and entero-toxigenic *E.coli* (ETEC) resulting in diarrhea at various ages with potential active transmissions to contacting humans. Antibiotics are frequently applied in veterinary medicine, but worries were elevated concerning antibiotic resistance through the native intestinal microbiota of animals **(Moyaert *et al.*,2005).**The current study, *K. pneumoniae* was isolated from 21% and 13% of dog and cat fecal samples, respectively.

A finding that highlights the possible role of dogs and cats as a reservoir of *K. pneumoniae* that could be dessiminated to people. A finding that agrees with those findings of **Marques *et al.* (2019).** *K. pneumoniae* could result in significant infections such as pneumonia. Therefore, upcoming comparative reports must comprise *K. pneumoniae* straining from more clinical origins to more understanding the role of dogs as reservoirs of infective *K. pneumoniae*. The *K. pneumoniae* clonal lineages noticed in dogs from the current work were formerly isolated from various kinds of human infection in addition to UTI. Therefore, pet clinicians must recommend good hygiene procedures and safe disposal of feces to minimize the potential for direct and indirect zoonotic transition of the microbes **(Cátia Marques *et al.*, 2019).**

However it is more important than the zoonotic potential of *E. coli* and *K. pneumoniae* infections in pets is the potential dissemination of antimicrobial resistant strains to people in contact. This is a marvel that was in fixed development since the presentation of antibiotics and numerous agents are identified to enhance bacterial resistances. The main enhancers for such marvel are failures of following treating regimens, prophylactic usage of antibiotics and the usage of antibiotics as growing stimulus in animals.

CONCLUSION

Analysis of data presented in the current study showed that *E. coli* and Klebsiella are considerd as the most common enteropathogenic associated with diarrhea in cats and dogs

with multiple virulence genes and antibiotic resistance that has public health hazards as it may act as a source of infection to human being.

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