



Original Research

Molecular Characterization and Detection of Antibiotic Resistance Genes in *E. coli* Isolated from Stray Dogs

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Abstract

This study investigated the presence of some virulence and antibacterial resistance genes in E. coli isolates from stray dogs. Antimicrobial resistance in Escherichia coli isolated from stray dogs can be considered a potential threat of infection for the human population. Particularly the peoples who are in close contact with such stray dogs are at the most risk. Our objective in the present study was to isolate the E. coli from the faeces of the stray dogs and to study the resistance pattern as well as the virulent gene associated with it. In present study, total 42 rectal swabs were collected aseptically in a sterile bottle and transferred to laboratory. Total 18 of the E. coli were isolated in all. Antibiotic sensitivity assay was conducted with total 10 different antibiotics and the results showed highest sensitivity towards Enrofloxacin (72%) followed by Ceftriaxome (63%), Ciprofloxacin (54%) Gentamycin (45%), and Chloramphenicol (36%). Multiple drug resistance was observed towards tetracycline (81%), Ampicillin (63%), Amoxicillin-clavulinic acid (36%), Penicillin (27%) and Streptomycin (27%). Almost all 18 of E. coli isolated from rectal swabs of dogs were resistant to one or more antimicrobial agents respectively. The multidrug resistant strains of E. coli isolates were further examined for expression of tet (A) and tet (B) genes with published primer sequence. Majority of multidrug resistant strains E. coli isolated expressed tet (A) gene in 11(61%) and tet (B) in 05(28%) isolates. From the E. coli isolates, virulent gene stx1 was expressed in 02 samples and stx2 was expressed in 05 samples. In conclusion, this study highlights the role of E. coli in stray dogs in terms of its virulent and antibiotic resistant genes.

Key words: Antibiotic Resistance, *E. coli*, Stray Dogs, Virulent Gene

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Introduction

Stray dogs are the free roaming animals which may reach the human localities and spread the infection through the faeces and may contaminate the water sources or the vegetation. Small children can acquire the infection while playing in the common parks which are mainly having free access to stray dogs. Infection introduced due to stray dog into such local areas leads many times to gastrointestinal problems in childrens. It is important to note that the bacterial ability to transfer genetic cassettes, which confer resistance to several classes of drugs, and observations of the spread of resistance have been seen increased in the recent years (Aleksium, M.N. 2007 and Carattoli, A. 2009). The molecular characterization of antimicrobial resistance could be very useful not only in surveillance studies, monitoring and tracking of multidrug-resistant strains but also in obtaining information about commonality among human and animal bacterial isolates.

Material and Methods

For the present study total 42 rectal swabs of stray dogs were collected directly in a sterile bottle with the help of sterile swab. The swabs were transferred to laboratory in a sterile condition and was processed for isolation of *E. coli*

Isolation and Identification of the *E. coli*

Selective Plating for Growth of *E. coli*

For selective isolation of *E. coli* the swab was inoculated in nutrient broth and incubated. After incubation a loopful of nutrient broth culture was streaked on MacConkey agar plate and incubated at 37°C for 24 hours. After incubation the individual lactose fermenting colony was selected and streaked on EMB agar plates and incubated for 37°C for 24hrs. Colonies showing characteristic metallic sheen on EMB agar were considered as presumptive *E. coli* isolates. Further the organisms were processed for biochemical typing and the isolates were examined for antibiotic sensitivity test by using disc diffusion technique.

Antibiogram Pattern of the Isolate

In-vitro antibiotic sensitivity pattern of the isolates were studied by using disc diffusion method (Bauer *et al.*, 1966) against ten commercially available antibiotic disc of known concentration (Hi-Media, Mumbai) viz. ampicillin(10µg), amoxicillin/clavulanic acid, ceftriaxone(30 µg), chloramphenicol(30 µg), ciprofloxacin(5µg), enrofloxacin(10 µg), gentamycin(10µg), penicillin(10units), streptomycin(10µg) and tetracycline(30 µg). Results were recorded after 18 hrs of incubation at 37°C, the diameter of zone of growth inhibition were interpreted as per manufacturer's instructions.

Molecular Detection of the Drug Resistance Gene and Virulent Gene

All *E. coli* isolates were further screened for detection of drug resistance and virulence genes by performing Polymerase Chain Reaction (PCR) on the purified DNA of organisms. *E. coli* isolated from faecal samples were subjected to Polymerase Chain Reaction (PCR) targeting specific genes viz. tetA, tetB, and stx1 and stx2 using method described by Momtaz *et al.*(2012) and Nguyen *et al.*(2010).

Purification and Extraction of Bacterial Genomic DNA

Isolation of DNA from *E. coli* was carried out by conventional boiling and snap chilling method (Wani *et al.*, 2003) with slight modifications. A single colony was inoculated in 1 ml nutrient broth and incubated at 37°C for 24 h. The cells were harvested by centrifugation at 5000 rpm for 10 min. The pellet was washed with normal saline by centrifuging at 500 rpm for 10 min for twice. Then, the pellet was resuspended in 500 µl normal saline solution and boiled for 10 min at 100°C and snap chilled on ice, after centrifugation at 1000 rpm for 5 min; 5 µl of supernatant was used as template for PCR reaction (Shrivani *et al.*, 2017).

PCR for detection of virulence genes stx1 and stx2

The DNA template obtained by boiling and snap chilling extraction technique was further subjected to PCR with specific primers (Table 1), for the detection of stx1 and stx2 in *E. coli*. PCR was performed by preparing final reaction volume of 20 µl in 0.2 ml thin walled PCR tubes. It was prepared by taking 10µl master mix supplied with Taq DNA, Mgcl2 and dNTPs; adding 1µM each of forward and reverse primers, 100ng template DNA and 5µl nuclease free. Samples were subjected to 35 PCR cycles, each consisting of Initial denaturation of 10 min at 94°C ;30 sec of denaturation at 94°C; 45 sec min of annealing at 50°C, and 1.30 min of elongation at 70°C.

Table 1: Agarose gel electrophoresis of PCR product

Gene	Primer and Oligonucleotide Sequence	Product Length	Reference
Stx1	(F)TTCGCTCTGCAATAGGTA	555bp	Nguyen <i>et al.</i> (2010)
	(R)TTCCCCAGTTCAATGTAAGAT		
Stx2	(F)GTGCCTGTTACTGGGTTTCTTC	118bp	Nguyen <i>et al.</i> (2010)
	(R)AGGGGTTCGATATCTCTGTCC		
tetA	(F) GGTTCACTCGAACGACGTCA	557bp	Randall <i>et al.</i> (2004)
	(R)CTGTCCGACAAGTTGCATGA		
tet B	(F) CCTCAGCTTCTCAACGCGTG	634bp	Randall <i>et al.</i> (2004)
	(R) GCACCTTGCTGATGACTCTT		

PCR for Detection of Drug Resistance Gene tetA and tetB

For the detection of drug resistance gene tet A and tet B, PCR was performed by using the specific primers (Table 1). PCR for amplification of tet A and tet B gene was prepared in a final reaction volume of 25 µl in 0.2 ml thin walled PCR tubes. It was prepared by taking 12.5 µl master mix supplied with Taq DNA,

Mgcl₂ and dNTPs; adding 1 µM each of forward and reverse primers, 100ng template DNA and 3.5 µl nuclease free water to make final volume of 25 µl. The amplification programme was subjected to 35 PCR cycles, each consisting of Initial denaturation of 10 min at 95°C ;1 min of denaturation at 94°C; 90 sec min of annealing at 55°C, and 1 min of elongation at 72°C. The final amplified PCR products were subjected for 1.5% agarose gel electrophoresis after staining with ethidium bromide ((5 µl /100 ml).

Results and Discussion

The presence of virulence and antimicrobial resistance genes in *E. coli* strains harbored by stray animals is of public health concern because humans are in close contact with these animals. In the present study, total 18(43%) *E. coli* were isolated from 42 rectal swabs of stray dogs. It is noteworthy that 07 (39%) of the 18 isolates examined, harboured stx gene in the healthy stray dogs. Koochakzadeh *et al.* (2014) reported 18.9% stx gene prevalence among 79 pathogenic *E. coli* isolates from a population of 252 canidae/equidae, this finding is quite less than the present study. The stx genes encodes shiga-like toxin (stx) also called verocytotoxin/verotoxin, a putative virulent factor involved in the pathogenicity of STEC also known as verocytotoxin-producing *E. coli* (VTEC) and EHEC strains (Paton and Paton, 1998; Goldwater *et al.*, 2012; Nguyen and Speradio, 2012; Shahrani *et al.*, 2014). The stx inhibits protein synthesis and allows invasion of the intestinal mucosa similar to what is observed in human shigellosis (Nguyen and Speradio, 2012). In this study detection of stx1 was (11%) whereas stx2 was 33% which suggest the dominance of stx2 gene in this region regarding the stray dogs (Fig.1 and Fig. 2).

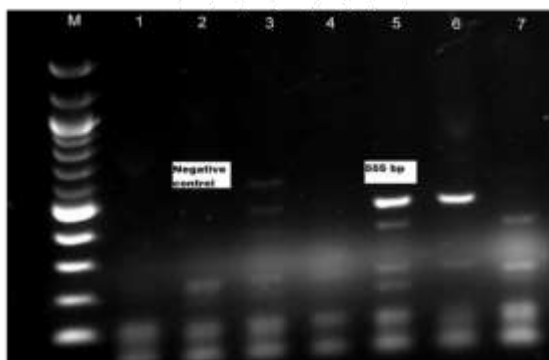


Fig1: Agarose gel electrophoresis of stx1 PCR products showing 555bp amplicon
Lane M- 100 bp Molecular ladder;
Lane 2:Negative control;
Lane 5 and 6:Positive for stx1

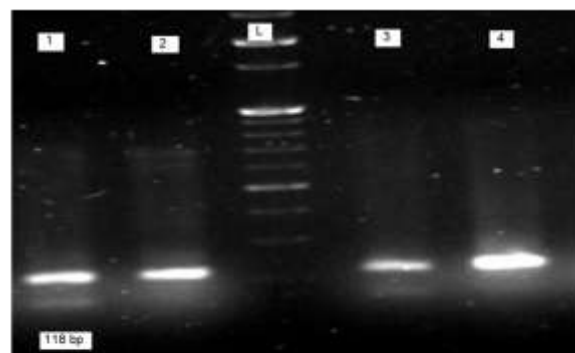


Fig 2: Agarose gel electrophoresis of stx2 PCR products showing 118bp amplicon
L-Marker Ladder of 100bp,
1,2,3 and 4-Positive for stx2

It is also higher than 3% STEC among 10 pathogenic *E. coli* isolates from 100 healthy/diarrhoeic dogs reported in Iran (Zahraei *et al.*, 2011). The interesting fact is that stx2 gene prevalence is more than stx1. Noticeably, in most studies, the prevalence of stx2 genes is greater than stx1 genes, as Beutin *et al.* (1997) reported that most of the cattle isolates were stx2 positive. The finding of high STEC (39%) prevalence in

this study, portends serious threat to public health since STEC strains causes highly fatal and untreatable infections such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) which causes renal failure in humans especially in children (Bentancor *et al.*,2007; Amisano *et al.*, 2011).

In the present study, multiple drug resistance was observed towards tetracycline (81%), ampicillin (63%), amoxicillin-clavulanic acid (36%), penicillin (27%) and streptomycin (27%). Strains showing high levels of resistance to ampicillin, streptomycin and tetracycline was also reported from faecal samples of pet dogs regardless of host species or group of origin (Carvalho *et al.*, 2016) Resistance to streptomycin, ampicillin, tetracycline, has been observed in other studies of dog faecal isolates (Pedreson *et al.*, 2007, Costa *et al.*, 2008 and Nam *et al.*, 2010). Aminopenicillins and aminoglycosides appear most often among the three classes of drugs with high frequencies of resistance in studies of samples of animal origin (Awaji, 2012 and Maynard, C. 2004). Highest sensitivity was observed towards enrofloxacin (72%) followed by ceftriaxome (63%), ciprofloxacin (54%) gentamycin (45%), and chloramphenicol (36%) and was comparable with findings of Wani *et al.*(2013) and Rehman *et al.* (2014) who reported higher sensitivity for STEC isolates from diarrheic calves to chloramphenicol and gentamycin antibiotics in Jammu and Kashmir of India. Multidrug resistant strain expressed *tet* (A) gene in 11(61%) of the *E. coli* isolates and *tet* (B) in 05(28%) isolates (Fig. 3).

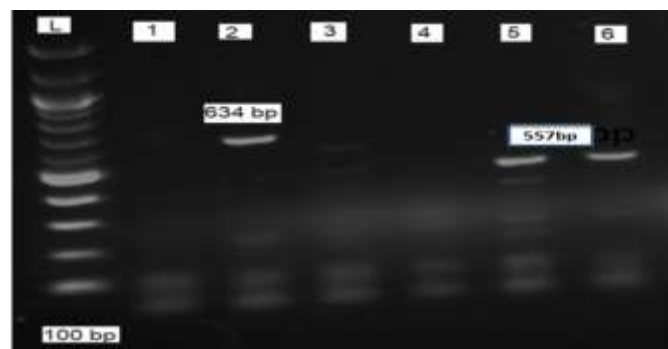


Fig:3 Agarose gel electrophoresis of *tetA* and *tetB* PCR products showing 557bp and 634bp amplicon:
Lane M- 100 bp Molecular ladder;
Lane 1:Negative control;
Lane:3 Positive for *tetA*;
Lane:5,6: Positive for *tetB*

The *tet(A)* and *tet(B)* genes are among several tetracycline determinants in *E. coli* which encode energy-dependent membrane-associated efflux proteins (Roberts, 2005). Detection of *tet(A)* in 11 (61%) of the examined isolates as against 5 (28%) for *tet(B)* suggested that *tet(A)* may be the predominant tetracycline resistance gene harboured by pathogenic *E. coli* colonising dogs in this particular area. Similar study performed on diarrhoeic dogs in Iran showed the occurrence of 35.7% *tet(A)* gene followed by 21.4% *tet(B)* gene (Torkan *et al.*,2016).

Conclusion

In conclusion, the present study reveals the occurrence of virulent and antibiotic resistance genes in *E. coli* isolated from stray dogs and this further constitute as a source of environmental contamination and possess a serious threat to spread to other animals as well as human beings who are close contact with such animals.

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References

1. Alekshun, M.N., Levy, S. B. 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell*.128:1037–1050.
2. Amisano G, Fornasero S, Migliaretti G, Caramello S, Tarasco V, Savino F. 2011. Diarrheagenic *Escherichia coli* in acute gastroenteritis in infants in North-West, Italy. *New Microbiol* 34, 45-51.
3. Bauer AW, Kirby WMN, Sherris JC and Trunk MN. 1966. *Am. J. Clin. Pathol.* 45:493.
4. Bentancor A, Rumi MV, Gentilinic Sardoy K, Irino A, Agostini, A Cataldi. 2007. Shiga toxin-producing and attaching and effacing *Escherichia coli* in cats and dogs in a high hemolytic uremic syndrome incidence region in Argentina. *FEMS Microbiol Lett* 267, 251-256.
5. Beutin L. 1999. *Escherichia coli* as a pathogen in dogs and cats. *Vet Res.* 30, 285-298.
6. Carattoli A. 2009 Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother.*53:2227–2238.
7. Carvalho AC, Barbosa AV, Arais LR, Ribeiro PF, Carneiro VC and Cerqueira AMF. 2016. Resistance patterns, ESBL genes, and genetic relatedness of *Escherichia coli* from dogs and owners. *Braz. J. Microbiol.* 47(1), 150-158.
8. Costa D, Poeta P, and Sáenz Y.2008 Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. *Vet Microbiol.*127:97-105
9. Goldwater PN, Bettelheim KA. 2012. Treatment of enterohemorrhagic *Escherichia coli* (EHEC) infection and hemolytic uremic syndrome (HUS). *BMC Med* 10, 12.
10. Koochakzadeh A T, Zahraei Salehi, B Nayeri Fasaei, M Askari Badouei. 2014. Detection of verotoxin (Shiga-like toxin)-producing and eae harboring *Escherichia coli* in some wild captive and domestic equidae and canidae. *Arch Razi Inst* 69, 157-163.
11. Momtaz.H, Rahimi E and Moshkelani S. 2012. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinary Medicine*, 57 (4): 193–197.
12. Nam HM, Lee HS and Byun JW. 2010. Prevalence of antimicrobial resistance in fecal *Escherichia coli* isolates from stray pet dogs and hospitalized pet dogs in Korea. *Microb Drug Resist.*16:75-79
13. Nguyen,Tan Duc., Thin Thanh Vo., and Hung Vu-Khac.2010. Virulence factors in *Escherichia coli* isolated from calves with diarrhea in Vietnam. *Journal of Vet Sci.*,12(2):159-164
14. Nguyen Y and Speradio V. 2012. Enterohaemorrhagic *E. coli* pathogenesis. *Front Cell Infect Microbiol* 2, 90.
15. Paton JC and Paton AW. 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clin Microbiol Rev* 11, 450-479.
16. Pedersen K, Pedersen K, Jensen H, Finster K, Jensen VF, Heuer OE.2007. Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. *J Antimicrob Chemother.*60:775-781



17. Randall LP, Cooles SW, Osborn MK, Piddock LJ and Woodward MJ. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty five serotypes of Salmonella enterica isolated from humans and animals in the UK. *J Antimicrob Chemother* 53, 208-216.
18. Rehman, M.U, Rashid M, Sheikh JA, Wani SA and Farooq S. (2014) Multi-drug resistance among shiga toxin producing Escherichia coli isolated from bovines and their handlers in Jammu region, India. *Vet. World*, 6(9): 655-658.
19. Shahrani M, Dehkordi FS and Momtaz, H. 2014. Characterization of Escherichia coli virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biolog Res.* 47, 28.
20. Shrivani M, Y. Narasimha Reddy, Subramanyam K.V, M. Ramakoti Reddy and T. Srinivasa Rao 2017. Prevalence and antimicrobial resistance pattern of Shiga toxigenic Escherichia coli in diarrheic buffalo calves. *Veterinary World.org/Vol.10/July-2017*
21. Torkan S. Bahadoranian MA. Khamesipour F. Anyanwu MU.2016. Detection of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from diarrhoeic dogs in Iran. *Arch Med Vet* 48, 181-190.
22. Wani SA, Bhat MA, Samanta I, Nishikawa Y and Buchh AS 2003. Isolation and characterization of shiga toxin-producing Escherichia coli (STEC) and enteropathogenic Escherichia coli (EPEC) from calves and lambs with diarrhea in India. *Lett. Appl. Microbiol.*, (37): 121-126.
23. Wani SA, Hussain I, Beg SA, Rather MA, Kabli ZA, Mir MA and Nishikawa Y. 2013. Diarrhoeagenic Escherichia coli and salmonellae in calves and lambs in Kashmir: Absence, prevalence and antibiogram. *Sci. Tech. Rev.*, 32(3): 1-17.
24. Zahraei Salehi T, Badouei MA and Gohari IM 2011. Molecular detection and antibacterial susceptibility of enteropathogenic Escherichia coli (EPEC) and shigatoxigenic *Escherichia coli* (STEC) strains isolated from healthy and diarrhoeic dogs. *Comp Clin Path* 20, 585-58.