Annals of Agri-Bio Research **27** (2) : 265-269, 2022 **Impact Score : 0.28 (Scopus)**

Antibiogram Profile of Diarrhoeagenic *Escherichia coli* Isolated from Canine

PAMELA SINGH, VIPIN KHASA*, SUNIL CHOPRA AND VIPUL CHAUDHARY

Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonipat-131 039 (Haryana), India
**(e-mail : vkhasasnp@gmail.com; Mobile : 98131 21824)*

(Received : March 10, 2022; Accepted : April 4, 2022)

ABSTRACT

One of the predominating bacteria that causes diarrhoea is *Escherichia coli (E. coli*) in canines and may need treatment. The present study was focused on the isolation of *E. coli* spp. from dogs; its morphological and biochemical characterization, and assessment of its antibiotic sensitivity. About 20.8% of *E. coli* were identified in 394 fecal stool samples of dogs collected from Haryana (India). Further, the age-wise distribution of isolates suggested that diarrhoea-causing *E. coli* was more frequently observed in dogs of more than one year age group (23.2%) followed closely by 0-3 months age group (21.2%). The present study demonstrated that almost 90% of sensitivity was observed to gentamycin, followed by chloramphenicol (89%), ofloxacin (85%) and tetracycline (84%). The isolates were found carrying resistance to ampicillin (87%), cephalexin (73%) and cefotaxime (68%) followed by amoxicillin/clavulanic acid, streptomycin and amikacin. It was concluded that bacterial diarrhoea in canine needed attention mostly in young puppies up to three months of age due to possible threat from viral enteric pathogens which lead to secondary bacterial infections along with low level of host immunity. Intervention of animal husbandry sector can prove to be a major tool for controlling emergence and spread of antibiotic resistance.

Key words : Canine, diarrhoea, *Escherichia coli*, antibiotics resistance

INTRODUCTION

Gastrointestinal infections are one of the most commonly identified problems in canines with clinical manifestations of acute diarrhoea, leading to death due to loss of body fluid. Numerous microbial agents are associated with acute gastroenteritis. The most common viral enteric pathogen affecting canine population is canineparvovirus (CPV) both in the developed world (Schiro *et al.*, 2022) and also in developing countries. *Escherichia coli* (*E. coli*) along with other Salmonella spp. and Campylobacter spp. are among the most commonly reported bacterial pathogen responsible for canine diarrhoea (Verma *et al.*, 2021). *E. coli*, a member of Enterobacteriaceae family, is a Gram-negative, non-sporulating, flagellated, rod-shaped and facultative anaerobic bacterium (Ranjbar *et al.*, 2017). As per estimate, [European Centre for Disease Prevention Control (ECDC) *et al.*, 2017; King *et al.*, 2018] antibiotics usage of livestock and companion animals was almost double to humans, although the decreased ratio has been observed in current years (ECDC *et al.*, 2017). The antimicrobial resistance (AMR) dissemination was linked with spread of antimicrobial-resistant bacteria between animals and humans (Chirila *et al.*, 2017). Acquired AMR mechanisms such as ESBLs have been found in *E. coli* conversing resistance to a number of antibiotics like cephalosporins and penicillins (Valat *et al.*, 2020). There is a requirement of assessment of cases of diarrhoea in canine for identification of pathogens and selection of effective antibiotics to be used for decreasing resistance problem. Considering the above, the present study was planned and performed for the identification and biochemical characterization of the bacterial causative agents of diarrhoeal infections caused by *E. coli* in canine in Haryana state and further to study, their antimicrobial resistance profiling to aid in the future treatment.

Presently : State Disease Diagnostic Laboratory, Department of Animal Husbandry, Sonipat-131 001 (Haryana), India.

MATERIALS AND METHODS

A total of 394 fecal samples from diarrheic dogs were presented for diagnostic analysis in Laboratory of DCR University of Science and Technology, Murthal, Sonipat, Haryana and Disease Diagnostic Laboratory, Animal Husbandry, Sonipat, Haryana. The isolation was performed from the year 2019 to 2020.

Swab samples were streaked on Mac Conkey's Agar (HiMedia Laboratory, India) and incubated for 24 h at 37°C to isolate the pure colony. These samples were identified by cultural morphology and biochemical characteristics. All colonies were stained by Gram's staining (HiMedia Laboratory, India) method. The Gram-negative bacilli suggestive of *E. coli* were further processed for biochemical characterization (Indole, Methyl Red, Vogus Proskauer, citrate, ONPG and lysine utilization) using *E. coli* identification kit KB010 (HiMedia Laboratory, India). The isolates were preserved at 4°C till further use.

All 82 *E. coli* isolates were processed for antibiogram profiling according to the disc diffusion method by using commercially prepared disc (Himedia, India) with the identified concentration of antibiotics. For antibiogram profiling, following antibiotics used were : Ampicillin (AMP) 10 mcg, Amoxyclav (AMC) 30 mcg, Amikacin (AK) 30 mcg, Ciprofloxacin (CIP) 5 mcg, Cefotaxime (CTX) 30 mcg, Cefalexin (CN) 30 mcg, Chloramphenicol (C) 30 mcg, Colistin (CL) 10 mcg, Enrofloxacin (EX) 5 mcg, Kannaycin (K) 30 mcg, Gentamicin (GEN) 10 mcg, Ofloxacin (Of) 5 mcg, Streptomycin (S) 25 mcg and Tetracycline (TE) 30 mcg (HiMedia, Mumbai, India). The spread culture of each isolate on Mueller Hinton agar plates was incubated overnight at 37°C, followed by an examination of the inhibition zones by examining as per the recommendations of Clinical and Laboratory Standards Institute 2017 (CLSI).

RESULTS AND DISCUSSION

Out of 394 fecal samples, a total of 82 (20.8%) were found positive for *E. coli*, while four (1%) were found infected with non-lactose fermenting Gram-negative bacteria (Table 1). The rest of the 308 samples (78.1%) were found negative for Gram-negative bacterial infection. Females (278) were found more infected with diarrhoea compared to male (116) dogs. Out of these isolates of *E. coli*, 24 (21.2%) were found in less than three month old puppies out of 113 cases, while 7 (16.3%), 9 (15.8%) and 42 (23.2%) belonged to age group 3-6 month old (n= 43), 6-12 month old (n = 57) and more than one year age (n = 181), respectively.

Antibiotic susceptibility tests of all *E. coli* isolates against 14 antibiotics were observed, where maximum sensitivity was recorded against gentamycin (90%), chloramphenicol (89%), ofloxacin (85%) and tetracycline (84%) in 74, 73, 70 and 69 isolates, respectively (Table 2). Other antibiotics against which sensitivity was detected in isolates were ciprofloxacin (79%), enrofloxacin (79%), colistin (78%), kanamycin (70%), amikacin (67%), streptomycin (67%) and amoxicillin/clavulanic acid (57%). Maximum resistance was found against ampicillin (87%), cefalexin (73%) and cefotaxime (68%) followed by amoxicillin/ clavulanic acid (43%).

In the present study, a significant proportion (20%) of diarrhoea cases were shedding *E. coli* bacterium in loose or watery feces. However, a large number of cases were found infected with pathogens other than of Enterobacteriaceae family.

The findings of Dhaka *et al.* (2016) on diarrhoea cases of animals indicated that *E. coli* contributed to 59 of 106 cases, which was partially in agreement with our study. Among urinary tract infections (UTI) in dogs, *E. coli* was infecting 24.3% of dogs in Haryana (Mustapha *et al.*, 2019) which was similar to

Table 1. Sex and age-wise distribution of cases

Age	Sex-wise case			*E. coli* isolated	Non-lactose fermenting bacteria	No bacterial cases
	Male	Female	Total			
0-3 months	40	73	113	24	0	89
3-6 months	18	25	43	7	1	35
6-12 months	3	54	57	9	0	48
More than 1 year	55	126	181	42	3	136
Total	116	278	394	82	4	308

Table 2. Antibiogram profiling of *E. coli* isolates from canine suffering from diarrhoea

S. No.	Name of antibiotic		No. of resistant	Per cent of resistant	No. of sensitive	Per cent of sensitive
1.	Amikacin	AK 30	27	32.9	55	67.1
2.	Amoxicillin/clavulanic acid	AMC 10	35	42.7	47	57.3
3.	Ampicillin	AMP 10	71	86.6	11	13.4
4.	Cefotaxime	CTX 30	56	68.3	26	31.7
5.	Cefalexin	CN 30	60	73.2	22	26.8
6.	Chloramphenicol	C 30	9	11.0	73	89.0
7.	Ciprofloxacin	CIP 5	17	20.7	65	79.3
8.	Gentamicin	GEN 10	8	9.8	74	90.2
9.	Streptomycin	S 25	29	35.4	55	67.1
10.	Tetracycline	TE 30	13	15.9	69	84.1
11.	Enrofloxacin	Ex 5	17	20.7	65	79.3
12.	Colistin	CL 10	18	22.0	64	78.0
13.	Kannamycin	K 30	15	18.3	57	69.5
14.	Ofloxacin	Of 5	12	14.6	70	85.4
	Total=82					

the present study on diarrhoea cases. There were chances that bacteria penetrated the urinary tract from the infected intestine of the animal. So, both studies suggested that although *E. coli* was a significant bacterial pathogen causing disease in dogs other agents like the viruses may also be responsible for morbidity in dogs in UTI and gastrointestinal cases. Among viral causes, canine parvovirus and adenovirus remained one of the main agents responsible for diarrhoea worldwide and in India (Behera *et al.*, 2015; Agnihotri *et al.*, 2017). A study from Orissa (eastern India) suggested that more canine male population (86.2%) was infected with canine coronavirus, on the contrary to the present study where the more diarrheic female (70.5%) were infected than male (29.5%) dogs and puppies in Haryana. Another study suggested more female puppies suffered from parvoviral diarrhea (Chethan *et al.*, 2020) which was partially in agreement with the present study. Among age-wise distribution in the present study, dogs aged more than one year old were found slightly more affected (23.2%) by *E. coli* diarrhoea than 3-month old puppies (21.2%), while the in between age group were found less affected. The main reason for this may be either weak immune status in youngsters and old age along with more outdoor activity routine in older dogs. Chethan *et al.* (2020) also recorded that less than 3-month old puppies were affected more (44%) by canine parvoviral diarrhoea than another age groups, which was partially in agreement with the present study. A similar finding from another study was reported where diarrhoea caused by viruses mostly affected less than 3-month old puppies (Duijvestijn *et al.*, 2016).

Although most of the diarrhoea cases recovered without antibiotics, still their use was in practice to avoid any causality in dogs because of lack of treatment (Candellone *et al.*, 2020). *E. coli* infected both humans and animals, which made it important to keep a check on infection in dogs to prevent the spread of infection. The antibiotic resistance problem was duly recognized by human and veterinary health professionals, for which there was a need for a collaborative effort. In India, the network started working by linking all stakeholders. Every effort and study can prove useful for keeping us ready for the present and future. In the present study, most of the isolates were sensitive to gentamycin, chloramphenicol, tetracycline and ofloxacin which suggested that there was scope for use of these drugs for therapeutic purposes. Still, in most of the cases in clinical practice, ofloxacin was the popular choice followed by gentamycin among veterinarians. This was in agreement with the study of Agnihotri *et al.* (2017) where sensitivity to chloramphenicol, amikacin and gentamycin was found encouraging for their use in dogs. Another study by Valat *et al.* (2020) found sensitivity against kannamycin in addition to the above three antibiotics. In the present study, resistance was recorded against ampicillin, cephalosporin (Cefotaxime and cefalexin) followed by amoxicillin/clavulanic acid, which

suggested that their use was more in practice and resistance could have developed. These were common drugs used for the treatment of canine diarrhoea, skin problem and respiratory problem. The problem with resistance against them was not limited to the treatment of dogs but also fear of the spread of this resistance among bacteria infecting humans around us.

Resistance against tetracycline, followed by ampicillin and cefotaxime, co-trimoxazole and ceftriaxone was found significantly higher in *E. coli* isolates of food origin (Dhaka *et al.*, 2016) from India. This study was similar to the finding of the present study which suggested that the current situation prevailed in the larger part. However, resistance to tetracycline reported from Japan (Hata *et al.*, 2022) was found in contrast to the present study where *E. coli* isolates were found sensitive against this antibiotic, suggesting some changes in therapeutic practice in canines. Multidrug resistant of *E. coli* infection in Italy (Mattioni Marchetti *et al.*, 2020) was found to be deadly for puppies on which treatment with amoxyclav and enrofloxacin was found ineffective, which was in partially contrast to present study where these enrofloxacin had fair level sensitivity but still resistance to several antibiotics was observed.

The importance of antibiotic sensitivity assays can be understood when the report of multidrug resistance in *E. coli* isolates of human host was recorded. The maximum resistance to cephalosporins and fluoroquinolones was reported in uropathogenic *E. coli* isolates of humans (Malik *et al.*, 2021). The similar resistance to cephalosporin of animal origin from the same region made it necessary to undertake more studies of epidemiological nature.

CONCLUSION

It was concluded that *E. coli* was one of the major gram negative bacterial pathogens responsible for diarrhoea in the canine population of the area, which mainly affected young population with native immunity and old age dog patients. Treatment with antibiotics was the primary veterinary intervention, however, resistance to drugs like cephalosporin and ampicillin, which were useful for humans suggesting that antibiotics should be used cautiously.

ACKNOWLEDGEMENTS

The study was completed with necessary support from Department of Animal Husbandry, Haryana and Department of Biotechnology, DCR University of Science and Technology.

REFERENCES

Agnihotri, D., Singh, Y., Maan, S., Jain, V., Kumar, A., Sindhu, N., Jhamb, R., Goel, P. and Kumar, A. (2017). Molecular detection and clinico-hematological study of Viral Gasteroenteritis in dogs. *Haryana Vet.* **56** : 72-76.

Behera, M., Panda, S. K., Sahoo, P. K., Acharya, A. P., Patra, R. C., Das, S. and Pati, S. (2015). Epidemiological study of canine parvovirus infection in and around Bhubaneswar, Odisha, India. *Vet. World* **8** : 33-37.

Candellone, A., Cerquetella, M., Girolami, F., Badino, P. and Odore, R. (2020). Acute diarrhea in dogs : Current management and potential role of dietary polyphenols supplementation. *Antioxidants* **9** : 725. *Doi : 10.3390/antiox9080725.*

Chethan, G. E., Singh, M., Chander, V., Singh, D., Rajesh, J. B., Prasad, H. and Kumar De, U. (2020). Occurrence of canine parvovirus-2 and canine adenovirus-1 infections in dogs : A hospital based study. *Ind. J. Anim. Res.* **55** : 217-221.

Chirila F, Tabaran, A., Fit, N., Nadas, G., Mihaiu, M., Tabaran, F., Catoi, C., Reget, O. L. and Dan, S. D. (2017). Concerning increase in antimicrobial resistance in Shiga toxin-producing *Escherichia coli* isolated from young animals during 1980-2016. *Microbes Environ.* **32** : 252-259.

Clinical and Laboratory Standards Institute (CLSI) (2017). M100Ed32 | Performance Standards for Antimicrobial Susceptibility Testing, 32nd edn. [WWW Document]. *n.d. URL https://clsi.org/standards/products/microbiology/documents/m100/ (accessed 3.16.22).*

Dhaka, P., Vijay, D., Vergis, J., Negi, M., Kumar, M., Mohan, V. and Rawool, D. B. (2016). Genetic diversity and antibiogram profile of diarrhoeagenic *Escherichia coli* pathotypes isolated from human, animal, foods and associated environmental sources. *Infect. Ecol. Epidemiology* **6** : 31055. *http://dx.doi.org/10.3402/iee.v6.31055.*

Duijvestijn, M., Mughini-Gras, L., Schuurman, N., Schijf, W., Wagenaar, J. A. and Egberink, H. (2016). Enteropathogen infections in canine puppies : (Co-) occurrence, clinical

relevance and risk factors. *Vet. Microbiol*. **195** : 115-122.

European Centre for Disease Prevention Control (ECDC). (2017). EUCAST : EUCAST [WWW Document]. *n.d. URL https:// www.eucast.org/ (accessed 3.16.22).*

Hata, A., Fujitani, N., Ono, F. and Yoshikawa, Y. (2022). Surveillance of antimicrobial-resistant *Escherichia coli* in sheltered dogs in the Kanto Region of Japan. *Sci. Rep*. **12** : 1-9.

King, L. M., Fleming-Dutra, K. E. and Hicks, L. A. (2018). Advances in optimizing the prescription of antibiotics in outpatient settings. *BMJ* **363**. *Doi : 10.1136/bmj.k3047*.

Malik, S., Rana, J. S. and Nehra, K. (2021). Prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. *Biomed. Biotechnol. Res. J.* **5** : 80-87.

Mattioni Marchetti, V., Bitar, I., Mercato, A., Nucleo, E., Marchesini, F., Mancinelli, M., Prati, P., Scarsi, G. S., Hrabak, J. and Pagani, L. (2020). Deadly puppy infection caused by an MDR *Escherichia coli* O39 blaCTX–M–15, blaCMY–2, blaDHA–1, and aac (6)-Ib-cr–positive in a breeding kennel in Central Italy. *Front. Microbiol***. 11** : 584. *DOI : 10.3389/fmicb.2020.00584*.

Mustapha, M., Goel, P., Kumar, V., Agnihotri, V., Kumar, T. and Maan, S. (2019). Isolation of uropathogenic *Escherichia coli* from dogs and molecular detection of chloramphenicol resistance genes. *Har. Vet.* **58** : 66-69.

Ranjbar, R., Masoudimanesh, M., Dehkordi, F. S., Jonaidi-Jafari, N. and Rahimi, E. (2017). Shiga (Vero)-toxin producing *Escherichia coli* isolated from the hospital foods, virulence factors, o-serogroups and antimicrobial resistance properties. *Antimicrob. Resist. Infect. Control* **6** : 01-11.

Schiro, G., Gambino, D., Mira, F., Vitale, M., Guercio, A., Purpari, G., Antoci, F., Licitra, F., Chiaramonte, G., La Giglia, M., Randazzo, V. and Vicari, D. (2022). Antimicrobial resistance (AMR) of bacteria isolated from dogs with canine parvovirus (CPV) infection : The need for a rational use of antibiotics in companion animal health. *Antibiotics (Basel)* **142.** *Doi : 10.3390/antibiotics11020142*.

Valat, C., Drapeau, A., Beurlet, S., Bachy, V., Boulouis, H. J., Pin, R. and Haenni, M. (2020). Pathogenic *Escherichia coli* in dogs reveals the predominance of ST372 and the human-associated ST73 extra-intestinal lineages. *Front. Microbial.* **11** : 580. *Doi : 10.3389/fmicb.2020.00580.*

Verma, A., Carney, K., Taylor, M., Amsler, K., Morgan, J., Gruszynski, K., Erol, E., Carter, C., Locke, S., Callipare, A. and Shah, D. H. (2021). Occurrence of potentially zoonotic and cephalosporin resistant enteric bacteria among shelter dogs in the Central and South-Central Appalachia. *BMC Vet. Res*. **17** : 313. *Doi : 10.1186/ s12917-021-03025-2*.