Haryana Vet. (June, 2024) **63(1),** 4-9 ISSN 0033-4359 (P) **Research Article**

ANTIBIOTIC RESISTANT ZOONOTIC *ESCHERICHIA COLI* AND *SALMONELLA* SEROTYPES IN DOGS-A CLINICAL STUDY

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ABSTRACT

Escherichia coli and *Salmonella* spp. are zoonotic pathogens affecting dogs leading to enteritis. Aim of the current investigation was to identify, characterize and determine antibiogram profiling of the two bacteria of zoonotic importance i.e., *E. coli* and *Salmonella* spp. that are responsible for causing enteritis in companion animals. A total of fifty faecal swab samples from dogs suffering with enteritis reported to VCC, LUVAS during the study period of 6 months were collected, identified, and characterized with the help of PCR, serotyping and antibiogram pattern was determined. *Salmonella Lindenberg*, an emerging zoonotic pathogen was isolated from dogs. To the best of our knowledge, this is the first report on the isolation of *S. Lindenberg* from dogs. Enterohaemorrhagic pathotypes of *E. coli* namely O120 and O145 were also isolated for the first time from dogs suffering from enteritis. Other serotypes of *E. coli* like O6, O11 and O8 were isolated with maximum prevalence of O83 *E. coli* serotype. *In vitro* antibiogram pattern of *E. coli* and *Salmonella* spp. isolates revealed the presence of multidrug as well as extreme drug resistance among the isolates of canine origin. With the isolation of these pathogens of zoonotic importance, it becomes imperative to work closely with their emergence and mode of transmission. Our study relates the occurrence of both these zoonotic bacterial isolates in pets and future challenges for treating such multidrug resistant zoonotic infections in pets as well as humans.

Keywords: Diarrheic dogs, *E. coli*, *Salmonella Lindenberg*, Multidrug resistance

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Enteritis, commonly manifested as diarrhea, is one of the most common maladies faced by the veterinarians or human clinicians. Enteritis in dogs is multifactorial and is caused by different bacteria, viruses and protozoans (Bodewes *et al.*, 2014). Among bacteria, *E. coli*, *Salmonella* spp., *Campylobacter* and *Clostridium* spp. are most common etiological agents of enteritis in dogs (Giacomelli *et al.*, 2015). Pets like dogs and cats can contribute significantly to the spread of pathogens in the home and environment by their excretions that can infect humans and other animals in the surrounding area (Galán-Relaño *et al.*, 2023). *E. coli* and *Salmonella* spp. are among the most important zoonotic pathogens and their presence in pets should be determined for better handeling these pathogens. Pets such as dogs that live in close vicinity of humans may act as potential reservoirs of antibiotic resistant strains of *E. coli* and *Salmonella* spp. (Johnson *et al.*, 2006). Antimicrobial resistance pattern among *Salmonella* and *E. coli* pathogens of pet animal origin is not well documented and understood. Moreover, World Health Organization (WHO) in 2017, published a list of antibiotic-resistant priority pathogens that includes *Salmonella* and *E. coli* and urged research and development agencies to focus on developing new antibiotics urgently and to regulate the usage of antibiotics in companion animals so that existing antibiotics can be used for better treatment of humans. So, the objective of the present study was to isolate, characterize and determine the *in vitro* antibiogram pattern of *E. coli* and *Salmonella* spp. isolates from pet dogs suffering from enteritis.

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MATERIALS AND METHODS

Isolation and identification of *E. coli* and *Salmonella* spp.

A total of fifty faecal swab samples were collected aseptically from rectum of dogs having enteritis reported at referral hospital, Lala Lajpat Rai University of Veterinary and Animal sciences, Hisar, Haryana, India. Culture characteristics on MacConkey agar (MLA), Eosin-methylene blue agar (EMB) and Brilliant green agar (BGA) were used for presumptive identification of *E. coli* and *Salmonella* spp. (Quinn *et al.*, 2004). Suspected colonies were subjected for Gram staining by using HiMedia Gram staining kit and observed under the oil immersion of the microscope for staining reactions. Additionally, biochemical testing was conducted using the HiMedia-India KB011 Hi-*Salmonella* TM identification kit and the HiMedia-India KB001-20KT Hi-*Enterobacteriacea* TM identification kit for *Salmonella* spp. and *E. coli*, respectively, in accordance with the manufacturer's instructions.

Molecular identification of *E. coli* and *Salmonella* spp. using Colony touch PCR assay

DNA was extracted from overnight enriched cultures. Extraction of DNA from the purified culture was carried out by snapchill method (Arora *et al.*, 2006). Extracted DNA was stored at -20°C for further amplification. Details of the oligonucleotide primers used for the amplification of 16S r*RNA* (Ziemer and Steadham 2003) or 23S r*RNA* (Riffon *et al.*, 2001) are illustrated in (Table 1). The amplified products were analyzed using 1% agarose gel electrophoreses gel and visualized under Gel DocTMEZ Image (USA).

Serotyping

After confirmation of isolates as *E. coli* and *Salmonella* spp., the isolates were sent to the National *Salmonella* and *Escherichia* Centre, Kasauli (H.P) for final biotyping and serotyping. Kauffman-White scheme was consulted and organisms were assigned to the *Salmonella* and *E. coli* serotype.

***In-vitro* antibiogram pattern**

Antimicrobial resistance pattern of the recovered isolates was established by performing disc diffusion method (Bauer *et al.*, 1966). The characterization of *Salmonella* spp. and *E.coli* strains as sensitive or resistant was based on the size of inhibition zone around the disc compared with the interpretation standards based on Clinical and Laboratory Standards Institute (CLSI, 2017) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) guidelines. For determination of antibiogram, a total of twenty (20) antimicrobials belonging to seven different antimicrobial groups based on their spectrum, frequency of usage and as per WHO guidelines were selected. Antibiogram results led to the characterization of isolates as multidrug resistant and extreme drug resistant. Bacterial isolates resistant to three or more antibiotics belonging to different antibiotic classes were placed under multidrug resistant category. Among multidrug resistant isolates, isolates sensitive to two or less than two antibiotics belonging to different groups were considered as extreme drug resistant (Schlegel *et al.*, 2012).

RESULTS AND DISCUSSION

Isolation and characterization of bacterial isolates

Out of total 50 rectal swabs collected from dogs suffering from enteritis, *E. coli* was recovered in 29 dogs (58%), whereas *Salmonella* spp. was isolated from six (12%) clinical cases (Table 2). The combination of culture characteristics on selective and differential media, Gram staining, biochemical testing, PCR assay and serotyping for the characterization of bacterial isolates were used. All the *E. coli* isolates on MacConkey Agar (MLA) showed typical pink lactose fermenting type of colonies and metallic sheen on EMB, while *Salmonella* spp. showed characteristic colourless non lactose fermenting colonies after twelve hours of incubation on MLA. *Salmonella* spp. showed red colonies surrounded by red halo on BGA and black coloured colonies on XLD. Confirmed positive cultures of *E. coli* and *Salmonella* spp. were initially characterized biochemically by using biochemical kit where *E. coli* showed + + - - IMVIC pattern and *Salmonella* spp. showed - + - + pattern. Results of biochemical test in the present study were in accordance

Table 1. Oligonucleotide primers used for the amplification of *E.coli* and *Samonella* spp. by colony touch PCR assay

Organism	Primers	Sequence	Product size
E. coli	Eco 223 (F)	ATCAACCGAGATT CCCCCAGT	232bp
	Eco 455 (R)	TCACTATCGGTCA GTCAGGAG	
Salmonella spp.	16Sr DNA (F)	TGTTGTGGTTAATA ACCGCA	574bp
	16Sr DNA (R)	CACAAATCCATCTC TGGA	

Table 2. Relative frequency of organisms isolated from selected dogs (n=50) suffering from enteritis on the basis of culture characteristics, biochemical testing and PCR

Organisms	No.	Percentage (%)
E.coli	29	58
Salmonella spp.	6	12
Other than *E.coli* and *Salmonella* spp.	15	30
Total number of isolates	50	

Table 3. Relative frequency of serotypes of *E. coli* isolates (n=23) and *Salmonella* spp. isolates (n=6) on the basis of serotyping from NSEC, Kasauli

Organism	Serotypes	No. of isolates	Percentage (%)
E.coli	O 120	5	21.8
	O 83	7	30.5
	UT	2	8.7
	O 145	1	4.3
	O 11	1	4.3
	O 8	1	4.3
	O 9	1	4.3
	Nonviable	5	21.8
Salmonella spp.	*Salmonella* Lindenberg	2	33.3
	Non-Viable/ Contaminated	4	66.6

Table 4. Antibiogram pattern of *E. coli* (n=29) and *Salmonella* spp. (n=6) isolates against 23 different antibiotics belonging to seven different classes

S.No.	Antimicrobials used		*E.coli* (n=29)		*Salmonella* spp. (n=6)	
			S%	R%	S%	R%
1.	Tetracyclines	Oxytetracycline	6(20.4%)	23(79.3%)	1(16.6%)	5(83.4%)
2.	Penicillins	Ampicillin	0	29(100%)	0	6(100%)
3.		Amoxicillin/10	0	29(100%)	0	6(100%)
4.		Amoxicillin/30	0	29(100%)	0	6(100%)
5.		Amoxicillin/clav	0	29(100%)	0	6(100%)
6.	Fluoroquinolones	Enrofloxacin	11(38.0%)	18(62.0%)	0	6(100%)
7.		Ofloxacin	8(27.6%)	21(72.4%)	2(33.3%)	4(66.7%)
8.		Moxifloxacin	10(34.5%)	19(65.5%)	1(16.6%)	5(83.4%)
9.		Levofloxacin	16(55.2%)	13(44.8%)	3(50%)	3(50%)
10.	Aminoglycosides	Gentamicin	25(86.2%)	4(13.8%)	4(66.7%)	2(33.3%)
11.		Amikacin	9(31.0%)	20(69.0%)	2(33.3%)	4(66.7%)
12.		Neomycin	11(37.9%)	18(62.1%)	4(66.7%)	2(33.3%)
13.		Kanamycin	16(55.2%)	13(44.8%)	1(16.7%)	5(83.3%)
14.	Cephalosporins	Ceftriaxone	16(55.2%)	13(44.8%)	4(66.7%)	2(33.3%)
15.		Cefotaxime	12(41.4%)	17(58.6%)	1(16.7%)	5(83.3%)
16.		Cefpodoxime	21(72.4%)	8(27.6%)	4(66.7%)	2(33.3%)
17.		Cefalexin	10(34.4%)	19(65.5%)	0	6(100%)
18.		Ceftriaxone/sulbactum	20(69%)	9(31.0%)	4(66.7%)	2(33.3%)
19.		Cefoperazone	12(41.4%)	17(58.6%)	3(50%)	3(50%)
20.	Chloramphenicol	Chloramphenicol	26(89.6%)	3(10.3%)	3(50%)	3(50%)
21.	Miscellaneous	Metronidazole	4(13.8%)	25(86.2%)	1(16.6%)	5(83.3%)
22.		Colistin	12(41.4%)	17(58.6%)	3(50%)	3(50%)
23.		Azithromycin	13(44.8%)	16(55.2%)	2(33.3%)	4(66.7%)

with the traditionally accepted characteristics of *E. coli* and *Salmonella* spp. with no variation in IMViC pattern as reported by many workers in the earlier studies (Bhargavi *et al.*, 2017; Kalambhe *et al.*, 2016). Pathogens other than *E. coli* and *Salmonella* spp. may also be responsible for causing enteritis in dogs as reported in the previous studies (Giacomelli *et al.*, 2015; Schlegel *et al.*, 2012). *Salmonella* shedding in the faeces was reported in healthy and dogs affected by canine parvovirus infection as 22% and 31%, respectively, which was higher than previous reports on young dogs, shelter dogs or dogs fed a raw meat diet (Botha *et al.*, 2018). Since the present study focused only on *E. coli* and *Salmonella* spp., so other prevalent pathogens were not characterized. Reporting of *E. coli* and *Salmonella* spp. pathogens in enteritis in dogs reflects poor hygienic conditions for pets. Defecation in open, raw, or semi-cooked meat to eat, stale home cooked food, contaminated water for drinking to pets may attribute to the occurrence of these pathogens.

Molecular identification of *E. coli* and *Salmonella* spp. isolates

All 29 isolates of *E. coli* was confirmed using specific primers and product size of approximately 232 bp was obtained. Similarly, six clinical isolates of *Salmonella* spp. were molecularly confirmed by amplified an expected product size of approximately 574 base pair (bp) using specific primers. Molecular techniques like PCR assay proved to be sensitive and specific in identification and confirmation of bacterial isolates like of *E. coli* and *Salmonella* spp. from other causative pathogens of bacterial enteritis in dogs (Ziemer and Steadham 2003).

Serotyping *E. coli* and *Salmonella* spp. isolates

Out of total 35 isolates i.e. 29 *E. coli* and 6 as *Salmonella* spp. isolates only 26 isolates i.e. 23 *E. coli* and 6 as *Salmonella* spp isolates were serotyped by CRI, Kasauli. Six different serotypes of *E. coli* were reported with maximum percentage of serotype O83 (30.5%) followed by serotype O120 (21.8%), O11, O6, O8 and O145. Two isolates of *Salmonella* spp. were identified as *Salmonella Lindenberg* which are of zoonotic importance and considered to be an emerging serotype (Table 3). Most frequently encountered serogroup of *E. coli* was O83 (7 isolates) which is considered as a normal inhabitant of GIT of dog, followed by O120 (5 isolates), O145 (1 isolate) belonging to the Shiga toxin producing *E. coli* pathotype. Serotypes O8, O9 and O11 belonging to the pathotype

enterotoxigenic *E. coli* were also identified. Serotype O83 was found to dominate among faecal and extraintestinal isolates from dogs and cats (Beutin, 1999), whereas in the past O145 was isolated from stray dog and coyote faeces (Jay-Russell *et al.*, 2014). Other pathotypes like O8, O6 and O11 were isolated from diarrhoeic and non-diarrhoeic dogs and human patients from different parts of world (Banik *et al.*, 2016). Enterotoxigenic pathotypes mainly O11, O6 and O8 of *E. coli* are responsible for causing diarrhea without any systemic signs in both dogs and human (traveller's diarrhea) (Coimbra *et al.*, 2000). In the current investigation, the moderate category of the canine hemorrhagic diarrhoea severity index (CHDSI) contained enterotoxigenic pathotypes of *E. coli*, specifically O8, O6, and O11, coupled with serotype O120 (EHEC pathotype), whereas the severe category contained serotype O145 (EHEC pathotype) of *E. coli*. To best of our knowledge this is the first report on the isolation of EHEC pathotypes O120 and O145 from dogs suffering with enteritis, whereas other serotypes such as O157, O101, O26, O153, O76 and O55 were recovered from enteritic dogs in the past (Banik *et al.*, 2016). Among different serotypes obtained in present study; O83, O120 and O145 have more importance in relation to human health. These may represent emerging serotypes in pets like dogs and reporting of these serotypes in pet may indicate a possible cross transmission between the pet owner and pet. These pets may act as reservoir for these pathogenic serotypes and as the pets live in proximity of humans, there are high chances of transmitting these pathogens to humans and environment or vice versa (Singh *et al.*, 2015).

Further, serotyping of *Salmonella* spp. revealed higher presence of the serovar *Salmonella Lindenberg* (33.35%). To the best of our knowledge, we report for the first time the isolation of *S. Lindenberg* from fecal samples of dogs having enteritis. The fact that dogs share common environment and are in close contact with humans has zoonotic implications. *Salmonella Lindenberg* is considered as a new emerging pathogen in human beings that is responsible causing non-typhoidal *Salmonella* infection globally (Alvarez *et al.*, 2004). Serotype *S. Lindenberg* has the same antigenic formula as serotype Typhimurium, except it has C2 serogroup O antigens. *S. Lindenberg* was isolated from 11.1% human patients mainly affected with disease conditions like systemic lupus erythematosus and chronic kidney disease (Sudhaharan *et al.*, 2018). Most recently *S. Lindenberg* was also isolated from 55.5% of edible frog meat samples obtained from a local market in Côte d'Ivoire and stated that it may be responsible for various health risks in humans consuming frog meat (Dadié *et al.*, 2017). Five different *S. enterica* serotypes i.e. *S. Anatum*, *S. Derby*, *S. Dublin*, *S. Infantis* and *S. Typhimurium* were reported from dog treats available in United Kingdom. It was stressed that the possible danger of zoonotic disease linked to these treats should be known by dog owners, pet food dealers, and veterinary practitioners. (Morgan *et al.*, 2023).

With the isolation of zoonotically important and new emerging serotypes of *E. coli* and *Salmonella* spp. from dogs it becomes utmost important to know more about the mode of transmission and other factors that may be helpful in establishing the control and therapeutic measures to combat these infections in both humans and pets like dogs.

***In vitro* antibiogram pattern**

The aim of identifying antibiogram was to determine the course of treatment and to generate a valuable data for future treatment plans. Antimicrobial resistance pattern of 29 *E. coli* and 6 *Salmonella* spp. isolates has been presented in Table 4. Overall resistance pattern irrespective of isolates, showed maximum resistance for penicillin and its derivative group (100%) followed by Metronidazole (85.7%), Oxytetracycline (80%), Ofloxacin and Cefalexin (71.4%), Moxifloxacin, Enrofloxacin and Amikacin (68.6 % each). Least resistance was found for Gentamicin and Chloramphenicol (17.1% each), Cefpodoxime (28.6%), Ceftriaxone sulbactum (31.4%) and Ceftriaxone (42.8%). Based on phenotypic *in vitro* sensitivity results in the present study it was found that all the 29 *E. coli* and 6 *Salmonella* spp. isolates were multidrug resistant. Among these multidrug resistant bacteria, 11 *E. coli* and 5 *Salmonella* spp. isolates were found to be sensitive to only 5 or 6 antibiotics of different groups and two *E. coli* and one *Salmonella* spp. isolates were extreme drug resistant i.e. only sensitive to two or less than two antibiotics belonging to different groups. The occurrence of resistance against *E. coli* isolates was highest to penicillins and its derivatives followed by tetracycline, and fluorquinolones. This study corresponds with the findings of Habib *et al.* (2016), wherein the authors also reported high level of resistance for ampicillin, sulphonamides, tetracycline and streptomycin in *E. coli*. Further, the findings related to the isolates sensitivity, these were found most sensitive for antibiotics such as Gentamicin, Chloramphenicol, Ceftriaxone-sulbactum which is in accordance with study of Maddison *et al.* (2008) who also reported sensitivity of *E. coli* isolates from diarrhoeic dogs against chloramphenicol and 3rd generation cephalosporins. For *Salmonella* spp. isolates, least sensitivity was observed towards Ampicillin, Amoxicillin/clavulanic acid, Enrofloxacin (0%) followed by Kanamycin, Moxifloxacin, Oxytetracycline, Cefotaxime,

Metronidazole (16.6%) and Amikacin, Ofloxacin, Azithromycin (33.3%). Higher level of sensitivity to Gentamicin, Chloramphenicol and 3rd generation cephalosporins such as Ceftriaxone sulbactum was in agreement with the findings of Dutta *et al.* (2008). The findings of this study points towards emerging resistance against many of the commonly used antibiotics, which may be because of lack of proper guidelines and polices for usage of important antimicrobials in dogs. It is intended from the study that more information is needed on antimicrobial resistance against bacterial pathogens of companion animals and its molecular basis in canine medicine, as well as on antimicrobial drug usage by veterinarians/pet parents in treating companion animals. By the practice of reducing the prescription of antimicrobials significant reduction in resistance in selected pathogens can be achieved (Prescott *et al.*, 2002). The antibiotic resistance in *E. coli* and *Salmonella* spp. has higher chances of getting transferred to commensals in gastrointestinal tract of dogs, as those pets can act as reservoirs for multidrug resistant pathogens and may contaminate environment.

CONCLUSION

The isolation of *Salmonella Linderberg*, an emerging pathogen for the first time from companion animals like dogs draws public health concern. Similarly, isolation of enterohaemorrhagic pathotypes of *E. coli* namely O120 and O145 for the first time from dogs suffering from enteritis in this study bring out the attention towards importance of pet hygiene and health care. The present study reflected the scenario of antibiotic resistance in the area with a matter of concern towards possible chances for transmission of antimicrobial resistance from pets to human beings. Therefore, serious efforts with regulatory policies in relation to usage of antimicrobials in pet health care need to implemented in veterinary clinical practice.

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Ethical considerations

Ethics waiver was obtained vide letter number VM/18/399, dated 15-05-2018, duly signed by Professor and Head Veterinary Medicine and submitted to Dean Post Graduates. Study is on clinical cases reported routinely at veterinary hospital of university and no ethical approval is required for such studies. The work is master's thesis work and thesis was approved by HOD, Veterinary medicine, Advisory committee of first author, external examiner and Dean Postgraduate studies. Verbal consent from the owner for processing the samples was taken.

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RETRACTION OF ARTICLE

This article earlier available at https://www.luvas.edu.in/haryana-veterinarian/download/harvet2016-dec/1.pdf entitled *"Occurrence of some organochlorine pesticide residues in poultry feed and meat"* has been retracted by the authors because of some error made during the data analysis process of the experimental observations due to counting the number of samples showing the concentration of pesticide below its corresponding Limit of Detection. All authors take full responsibility for this mistake and sincerely apologize for any inconvenience it may cause.

Editors