ISOLATION AND CHARACTERIZATION OF MULTI-ANTIBIOTIC-RESISTANT *E. COLI* FROM DIARRHOEIC COMPANION DOGS

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ABSTRACT

A study was undertaken to investigate the virulence potential and antibiotic resistance patterns of *E. coli* isolated from diarrheic companion dogs. The haemolysin production was recorded in 85.71% isolates while 92.85% isolates were shown to possess CR binding ability. Both these characteristics were recorded in 85.71% isolates. *In vitro* antibiotic sensitivity test revealed multiple antibiotic resistances developed in these isolates. The results alarm the potential public health hazards of *E. coli* from companion dogs.

KEY WORDS: *Escherichia coli*, dogs, antibiogram, haemolysin, Congo red assay

INTRODUCTION

Escherichia coli are considered as the normal inhabitant flora in the intestines of mammals and birds. Some *E. coli* strains are pathogenic due to the acquisition of virulence factors. The virulent *E. coli* are often associated with production of enterotoxin, verotoxin, colicins, siderophores, type-1 pili and motility and often show resistance to the lytic action of the host complement and antibiotics (Dho and Lafont, 1984; Chulasiri and Suthienkul, 1989). The dogs are preferred companion animals and considered as one of the family members in cities. The pathogenic strains of enteric *E. coli* from dogs are recognized as enterotoxigenic (ETEC), enteropathogenic (EPEC) and verotoxigenic (VTEC) based on their virulence properties. These pathogenic strains are associated with intestinal infections causing diarrhea as well as extra intestinal infections (Hammermueller *et al*., 1997 and Sancak *et al*., 2004). The infection spreads through direct contact and contaminated food and water (Zweifel *et al*., 2010).

The infection may also transmit to apparently healthy companion dogs. Its zoonotic potential with emergence of multiple-antibiotic-resistance has become challenging to veterinary and medical medicine for its control. Therefore, isolation of *E. coli* prevailing in companion dogs suffering from diarrhea and their characterization is important to reveal their pathogenicity. Hence, the present research was undertaken to isolate and characterize *E. coli* from clinical diarrheic cases of companion dogs.

MATERIALS AND METHODS

A total 48 faecal samples from diarrheic dogs were collected aseptically and brought to the laboratory during December 2012 to November 2013. The samples were inoculated in nutrient broth and incubated at 37°C for 12 hrs and streaked on Mac Conkey's agar for primary isolation. The lactose fermenting pink colonies were streaked on Eosin methylene blue agar for selective isolation of *E. coli*. The colonies having greenish metallic sheen were confirmed as *E. coli* and were subjected for morphological, cultural and biochemical characterization following the method of Cruickshank *et al*. (1975).

The *E. coli* isolates were screened for virulence potential pertaining to hemolysin production on 5% sheep blood agar. The Congo red (CR) binding assay was used as a phenotypic marker to distinguish between virulent and avirulent strains of *E. coli* (Burkhoff and Vinal, 1986). The black colored colonies with a dry crystalline consistency and weak slime producer pink colonies with an occasional darkening at the centers were selected for antibiotic resistance study.

The *E. coli* isolates were subjected to *in vitro* antibiotic sensitivity test by disc diffusion method as described by Bauer *et al*. (1966) using 30 mcg each of chloramphenicol, ampicillin/sulbactam , doxycycline, amoxicillin, neomycin and erythromycin (15 mcg) and ampicillin/cloxacillin (10 mcg), HiMedia discs. The diameter of zones of inhibition was measured to nearest millimeter and interpretation was made as described by the manufacturer.

RESULTS AND DISCUSSION

Out of 48 diarrhoeic faecal samples screened, 14 (29.16%) samples were found positive for E. *coli isolates* ,Which are gut acting, as reported by Olson *et al*. (1985) and Zweifel *et al*. (2010).

To determine the pathogencity of *E. coli* isolates, both hemolytic pattern and CR binding ability were studied. Out of 14 isolates of *E. coli*, 12 isolates (85.71%) were found positive for production of haemolysin. These findings of hemolytic test are in concurrence with Elseisy *et al*. (2010). Similarly 13 (92.85%) isolates were shown to possess CR binding ability. Both these characteristics were recorded in 12 (85.71%) isolates. The present study revealed CR binding by *E. coli* after 72 hours incubation at 37^0C. 7 isolates were grown as pink colour colonies indicating weak biofilm producers while 6 isolates showed black colour colonies indicating strong biofilm producers. One isolate appeared negative by this assay. Such findings are in concurrence with Parul *et al.* (2014).

The *in vitro* antibiotic sensitivity pattern of *E.coli* isolates is depicted in Table No.1.

Table No.1 *In vitro* antibiotic sensitivity pattern of *E.coli* isolates

Name of antibiotic	No. of isolates showed sensitivity	Sensitivity %	Resistance %
Ampicillin/Cloxacillin	8 (14)	57.14	42.86
Chloramphenicol	6 (14)	42.85	57.72
Ampicillin/Sulbactam	5 (14)	35.71	64.29
Ceftrizone	2 (14)	14.28	85.72
Amoxycillin	1 (14)	7.14	92.86
Erythromycin	1 (14)	7.14	92.86
Neomycin	1 (14)	7.14	92.86
Doxycycline	0 (14)	0.00	100

The *in vitro* antibiotic sensitivity pattern of all 14 *E. coli* isolates indicated maximum (57.14%) sensitivity towards Ampicillin/Cloxacillin, while 42.85% and 35.71% isolates were found sensitive to Chloramphenicol and Ampicillin/Sulbactam, respectively. The isolates showed high resistance towards Doxycycline (100%) followed by Amoxycillin, Neomycin and Erythromycin (92.86) and Ceftrizone (85.72%). These findings are in agreement with the findings of Tanvir *et al.* (2011) and Parul *et al*. (2014).

The findings indicated the haemolytic and biofilm producing properties along with multiple antibiotic resistance of *E. coli* isolated from the companion dogs. This may suggest that the companion dogs may become potential source of major public health hazards.

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