

## ISOLATION, IDENTIFICATION, PATHOTYPING AND ANTIBIOGRAM OF *ESCHERICHIA COLI* FROM RECTAL SWAB OF PET DOGS AND CATS

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**ABSTRACT:** Two hundred seventy two rectal swabs were examined from diarrhoeic and non-diarrhoeic pet dogs and cats. Out of 240 samples from dogs, 131 (54.58%) and out of 32 samples from cats, 15 (46.87%) were positive for *E. coli* by cultural characteristic, gram staining, motility, biochemical reactions and sugar fermentation tests. Out of 146 total positive isolates, only 12 isolates were pathogenic. Out of 12 pathogenic isolates of *E. coli* 6 isolates were O group non- typable and rest 6 *E. coli* strains were O25, O86a, O44 and O1 from dogs and O36 and O8 from cats. Out of 12 isolates 9 were sensitive to ceftriaxone, 8 were sensitive to ciprofloxacin and gentamicin, 4 were sensitive to amikacin, neomycin and norfloxacin, 2 were to Co-trimoxazole and 1 was sensitive to lomefloxacin. None of the isolate was sensitive to amoxyclav & cephotaxime.

**Key words:** *E.coli*, Pet dogs, Cats, Serotyping, Antibiogram.

### INTRODUCTION

*E. coli* is a normal habitat of the gastrointestinal tract of pet dogs and cats. But variants of this species are also amongst the important etiological agents of intestine and several extra-intestinal diseases. In newborn puppies the disease may be so acute that the only symptoms before death are weakness and inappetance even whole litters may be affected as "fading

puppy disease". To control zoonotic enteric diseases both in animal and human beings, broad spectrum antibiotics are used indiscriminately, which lead to antimicrobial resistance in enteropathogens like *E. coli*. The present study was designed to find out the involvement of *E. coli* in pet dogs and cats from apparently healthy and diseased animals along with their pathogenicity and antimicrobial sensitivity pattern.

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## MATERIALS AND METHODS

### Collection of samples

Out of two hundred seventy two rectal swabs, 240 were collected from pet dogs and 32 were from pet cats. Out of 240 rectal swabs collected from pet dogs, 60 samples were from diarrhoeic cases and 180 samples were from non-diarrhoeic cases. Out of 32 rectal swabs collected from pet cats, 8 samples were from diarrhoeic cases and 24 samples were from non-diarrhoeic cases.

### Culture of the organism

The individual swabs were dipped into *E. coli* broth for *E. coli* isolation and incubated for 18-24 hours at 37° C and then plated on MacConkey's agar and incubated at 37° C for 18-24 hours and then from lactose positive single colony culture was plated in Eosin Methylene Blue (EMB) and incubated at 37° C for 24 hours.

### Identification of isolates

The organism was identified on the basis of cultural characters, morphology (gram staining), and motility test and biochemical reactions according to Cruickshank *et al.* (1975). The organisms were tested for indole, methyl red, Voges-Proskauer, citrate utilization, nitrate reduction, triple sugar iron test and sugar fermentation tests.

### Pathogenicity test

According to Gupta and Singh (1969) adult albino mice were divided *i.e.* 4 mice in each group. One group of mice was inoculated intra-peritoneally (I/P) with 0.1 ml of inoculum (each isolate was incubated at 37° C for 16-18 hours in 5 ml of sterile nutrient broth fresh culture)

and control group also inoculated with 0.1 ml sterile nutrient broth in the same route and examined every 6 hours interval for 4 days. Mortality was recorded in each case. Heart blood and liver was collected for re-isolation of test organisms from the dead mice.

### Serotyping of the isolates

Serotyping of the isolates was performed at "National Institute of Cholera and Enteric Diseases" (NICED) in Kolkata.

### Antibiogram of different isolates

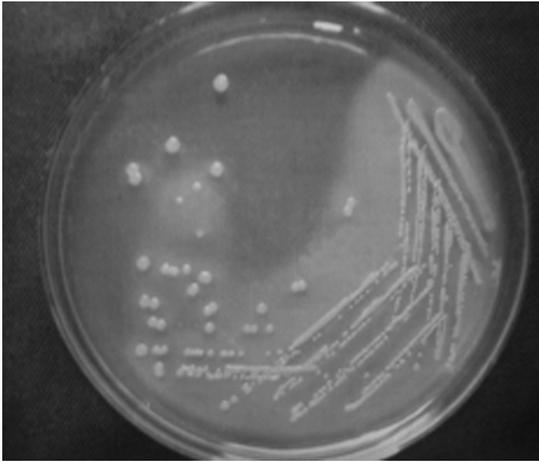
Antibiogram was carried out only with the pathogenic strain of *E. coli*. These isolates were tested against 10 different antimicrobial agents by using the discs diffusion technique described by Cruickshank *et al.* (1975).

## RESULTS AND DISCUSSION

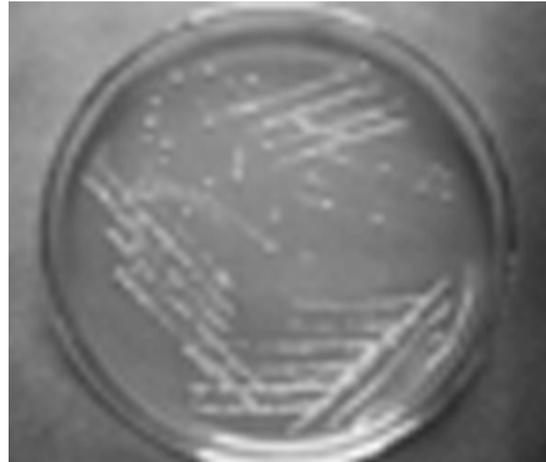
Out of the total 272 rectal swabs, 240 were collected from pet dogs and 32 from pet cats. *Escherichia coli* was isolated from 131 (54.58%) pet dogs and 15 (46.87%) pet cats.

Out of 240 rectal swabs collected from pet dogs 60 samples were from diarrhoeic cases and 180 samples from non-diarrhoeic cases. Out of 60 diarrhoeic pet dogs, 39 (65.0%) cases were found positive for *E. coli* and out of 180 non-diarrhoeic pet dogs, 92 (51.11%) cases were found positive for *E. coli*.

Out of 32 rectal swabs collected from pet cats, 8 samples were from diarrhoeic cases and 24 samples from non-diarrhoeic cases. Out of 8 diarrhoeic pet cats, 5 (62.5%) cases were found positive for *E. coli* and out of 24 non-diarrhoeic pet cats, 10 (41.66%) cases were found positive for *E. coli*. The isolation of *E. coli* was in agreement with the findings of



**Fig.1: Showing lactose-fermenting (pink colour) colonies of *E. coli* on MacConkey's**



**Fig.2: Showing colonies of *E. coli* with metallic sheen on EMB agar plate.**

Shouman *et al.* (1981), El-Gohary and Samaha (1992), Kucera *et al.* (1992) and Manna (2002).

All the isolates showed pink colour (lactose positive) colonies on MacConkey's agar (Fig. 1) and were plated on EMB agar. The isolates produced smooth, dark, blue-black colour with a typical metallic green showed on it after 18-24 hours of incubation at 37° C (Fig. 2).

All the isolates from EC broth were stained by Gram's staining method and examined under microscope and found gram-negative, stout rod. All the isolates were tested for motility by hanging drop method and found motile with different degree of movement *i.e.* sluggish to vigorous.

It was revealed that 100% isolates were positive to indole, methyl red and 100% negative to Voges-Proskaur. Twelve (9.16%) isolates from dogs and one (6.66%) isolate from cat utilized citrate. Manna (2002) obtained

citrate utilizing variant of *E. coli*. Ten isolates (7.63%) from dogs did not reduce nitrate. All the isolates from cat reduced nitrate. Mukherjee (1999) observed that 10.42% of *E. coli* isolated from piglets did not reduce nitrate. Manna (2002) also reported that 10.68% *E. coli* isolated from stray and household dogs did not reduce nitrate. In triple sugar iron (TSI) test, total 98.63% isolates of *E. coli* showed yellow butt (acidic) and yellow slant (acidic) but rest 1.37% showed variable results. Similar findings have also been reported by Edward and Ewing (1972) and Manna (2002). All isolated *E. coli* utilized glucose and lactose and produced acid and which were similar with the finding of Mukherjee (1999).

Pathogenicity test of all isolates was done in adult albino mice. Among the isolates 12 were found pathogenic. Out of 12 pathogenic isolates 3 isolates were highly pathogenic, 5 were moderately pathogenic and rest 4 were weakly pathogenic. Re-isolation of *E. coli* from

Isolation, Identification, Pathotyping and Antibiogram of *Escherichia coli*

**Table 1: Pathogenicity of *E. coli* isolates in albino mice.**

Isolates	Serotypes	No. of mice died within hours			Total no. died	% of death	Remark
		0-18	19-30	31-96			
E-35 <sup>b</sup>	O8	2	2	-	4	100	++
E-11 <sup>a</sup>	ONT	-	2	1	3	75	+
E-17 <sup>b</sup>	O36	2	1	-	3	75	++
E-76 <sup>a</sup>	O44	3	1	-	4	100	+++
E-47 <sup>b</sup>	ONT	-	2	-	2	50	+
E-50 <sup>d</sup>	ONT	1	2	-	3	75	++
E-54 <sup>a</sup>	ONT	1	1	1	3	75	+
E-83 <sup>a</sup>	O86a	3	1	-	4	100	+++
E-88 <sup>a</sup>	O25	3	1	-	4	100	+++
E-08 <sup>a</sup>	O1	1	2	-	3	75	++
E-67 <sup>c</sup>	ONT	-	1	2	3	75	+
E-92 <sup>b</sup>	ONT	1	2	1	4	100	++

a= Diarrhoeic dog

b= Diarrhoeic cats

c= Non- diarrhoeic dog

d= Non- diarrhoeic cat

+++ = Highly pathogenic

++ = Moderately pathogenic

+ = Weakly pathogenic

**Table 2: Antimicrobial drug sensitivity and resistance pattern of *E. coli* isolates (12nos.).**

Sl.No.	Name of the antimicrobial agents	Sensitive strains		Intermediate strains		Resistance strains	
		No.	%	No.	%	No.	%
1.	Amikacin	4	33.33	7	58.33	1	8.33
2.	Amoxyclav	0	0.0	4	33.33	8	66.66
3.	Ceftriaxone	9	75.0	0	0.0	3	25.0
4.	Cefotaxime	0	0.0	8	66.66	4	33.33
5.	Ciprofloxacin	8	66.66	0	0.0	4	33.33
6.	Co-trimoxazole	2	16.66	0	0.0	10	83.33
7.	Gentamicin	8	66.66	1	8.33	3	25.0
8.	Lomefloxacin	1	8.33	3	25.0	8	66.66
9.	Neomycin	4	33.33	5	41.66	3	25.0
10.	Norfloxacin	4	33.33	4	33.33	4	33.33

**Table 3: Multi resistance pattern of *E. coli* isolates (12 nos.).**

No. of antimicrobial agents	Resistance <i>E. coli</i> isolates		
	Number of Isolates	Serotypes	%
1	2	O36, ONT	16.66
2	3	O1, ONT, ONT	25.0
3	2	O86a, ONT	16.66
4	2	O25, ONT	16.66
8	1	O44	8.33
9	2	ONT, O8	16.66

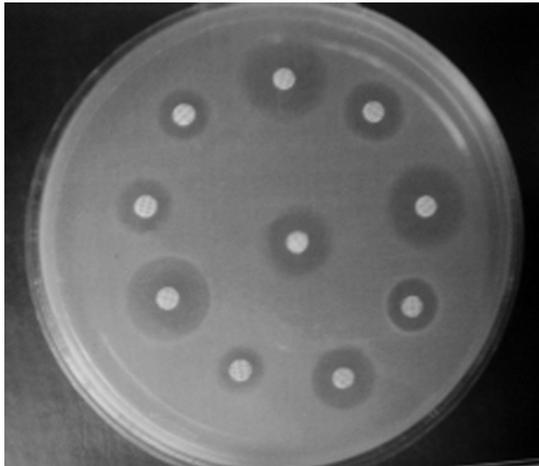
heart blood and liver of the dead mice fulfilled Koch's Postulates and confirmed the death of mice due to *E. coli* and this is in agreement with the findings of Manna (2002). During postmortem examination of the dead mice, internal organs like lungs, liver, spleen were found congested which were similar to the observation of Satyanarayana (1962).

Among the 12 pathogenic *E. coli* isolates only 6 were typed and rest 6 were 'O' group non-typable. Out of 6 typed *E. coli* 4 isolated strains *i.e.* O25, O86a, O44 and O1 were isolated from diarrhoeic cases of dogs and O36 and O8 were isolated from diarrhoeic cases of cats those were in agreement with Richter and Hellmann (1985) and Jain *et al.* (1991) who isolated the same strains of *E. coli* from diarrhoeic cases of dogs and cats. Pandey *et al.* (2003) isolated *E. coli* (enteropathogenic) *i.e.* O86a from diarrhoeic patients in Kolkata. Paciorek (2002) and Tamaki *et al.* (2005) isolated O44 strain from infantile diarrhoea in Poland, Kaneko *et al.* (2001) isolated enterotoxigenic *E. coli i.e.* O25 in Japan. Oswald *et al.* (2000) isolated entero-haemorrhagic *E. coli i.e.* O8 in human. Blanco

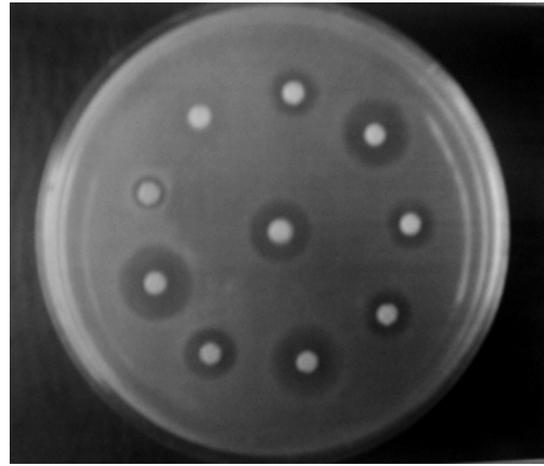
*et al.* (1996) and Kurazono *et al.* (2003) isolated uropathogenic *E. coli i.e.* O1 strain from companion animal like dogs and cats shared human uropathogenic *E. coli* which concluded human beings and companion animals share common O serotypes of pathogenic *E. coli* and cause severe disease in human and animals.

Twelve pathogenic isolates of *E. coli* were tested against 10 antimicrobial agents. Out of 12 isolates, 9 isolates (75%) were sensitive to ceftriaxone, 8 isolates (66.66%) were sensitive to ciprofloxacin and gentamicin, 4 isolates (33.33%) were sensitive to amikacin, neomycin and norfloxacin, 2 isolates (16.66%) were sensitive to co-trimoxazole and 1 isolate (8.33%) was sensitive to lomefloxacin. None of the isolate was found sensitive to amoxycylav and cephotaxime.

Out of 12 isolates, 8 isolates (66.66%) were sensitive to cefotaxime, 7 isolates (58.33%) to amikacin, 5 isolates (41.66%) to neomycin, 4 isolates (33.33%) to amoxycylav and norfloxacin, 3 isolate (25.0%) to lomefloxacin and 1 isolate (8.33%) to gentamicin were intermediately sensitive. None of the isolates



**Fig.3: Showing in-vitro sensitivity of *E. coli* (01) to different anti-microbial drugs (sensitive to amikacin, ceftriaxone, ciprofloxacin, co-trimoxazole, gentamicin, neomycin, norfloxacin; moderately sensitive to cefotaxime, lomefloxacin and resistant to**



**Fig.4: Showing in-vitro sensitivity of *E. coli* (08) to different anti-microbial drugs (sensitive to ceftriaxone, ciprofloxacin and gentamicin; moderately sensitive to amikacin, cefotaxime, lomefloxacin, neomycin, norfloxacin and resistant to amoxyclav, co-trimoxazole).**

was intermediately sensitive to ceftriaxone, ciprofloxacin and co-trimoxazole which are in accordance to Boro *et al.* (1983) and Manna (2002).

It was also revealed that out of 12 *E. coli* isolates, 10 isolates (83.33%) showed multiple drug resistance (from table 6). Two (16.66%), 3 (25.0%), 2(16.66%), 2(16.66%), 1 (8.33%) and 2 (16.66%) isolates were resistant against 1 (amikacin), 2(amoxyclav), 3(ceftriaxone), 4 (cefotaxime), 8 (lomefloxacin) and 9 (neomycin) respectively, which were accordance with finding of Awad-Masalmeh *et al.* (1990) and Manna (2002). The isolated *E. coli* were resistance to neomycin which might be due to indiscriminate use of antimicrobial agent (Awad-Masalmeh *et al.* 1990). Once multiple drug resistant organisms develop, they can persist in the host or in the

environment that is of public health importance since companion animals like dogs and cats act as reservoir of certain *E. coli* strains which were pathogenic to man (Kurazone *et al.* 2003).

## CONCLUSION

*Escherichia coli* is an important pathogen which causes severe health problem in dogs and cats. Serotyping result revealed that human beings, dogs and cats harbors some same 'O' serogroup of *E. coli*, so the disease can be shared between human and their companion animals like dogs, cats and sometimes it would be fatal to both human and animals. The infection can be avoided easily by proper hygienic precaution and judicious treatment which will avoid development of antibiotic resistant strains of *E. coli*.

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