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Pathogenecity of *E. coli* in mice isolated from fecal samples of zoo animals

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Abstract

Objectives: Certain strains of *E. coli* produce potentially lethal toxins. Food poisoning caused by *E. coli* can result from eating unwashed vegetables or poorly butchered meat which delivered to Zoo animals.

Materials and Methods: A total of 62 samples were collected aseptically and subjected to primary isolation by propagating in nutrient broth followed by culture on different agar media. Gram's staining and hanging drop techniques were also performed. Biochemical properties of the isolates were studied. Pathogenicity of 50 representative *E. coli* isolates were determined by lethality assay in adult mice models. *E. coli* was isolated successfully from 27 Samples out of 31 samples collected from seven species of animals of Rajshahi Zoo and 22 samples out of 31 samples collected from seven species of animals of Dhaka Zoo. All the *E. coli* isolates were found to produce bright pink colonies on MacConkey agar, yellowish green colonies surrounded by an intense yellow green zone on Brilliant Green (BG) agar and characteristic metallic sheen colonies on the Eosine Methyline Blue (EMB) agar. In Gram's staining technique, all the isolates were pink coloured, indicating Gram negative small bacilli while in the hanging drop technique the organisms were motile.

Results: All the *E. coli* isolates fermented dextrose, maltose, lactose, sucrose and mannitol with the production of both acid and gas. The results of catalase, MR and indole tests with *E. coli* isolates were positive but V-P test was negative. In the mice lethality assay, 5 *E. coli* isolates were virulent causing 10% death of the mice.

Conclusion: After mice lethality assay five pathogenic E. coli mainly isolated from carnivore animals in Dhaka zoo and Herbivore animals in Rajshahi zoo. Among five pathogenic E. coli, three of them found from Rajshahi zoo animals and two from Dhaka zoo animals.

Keywords: *Escherichia coli*, PCR, pathogenicity, zoo animal.

Introduction

Zoo or zoological garden in Bangladesh, serve as an useful public garden or park for community amusement as well as educative place particularly for children. It also provide provocating source of knowledge, love and affection to animals and birds of various kind of harbivorous and carnivorous classes. Thus, maintenance of health of such animals as well as birds are very crucial because of the nature of change of environment and way of living to which those specimen of animal kingdom are put to depriving their normal habitat and food.

While dealing with the health of such zoo-animals including birds proper and correct treatment of gastro-intestinal disorders is a common event faced with. In many-ocassion, drug resistance naturally of *E. coli* remains a matter of concern and as such improper use of antibiotic are often resorted to as a result.

The zoo animals and birds being dear, precious and charming, any ailment becomes crucial, asking immediate attention and care. With the discovery of many drugs, particularly that of antibiotic doctors very often are put to puzzling and perplexing situations and resort to indiscriminate use of such a panacea.

Animals kept at the zoo are usually bred in captivity, acquired from other facilities or captured in the wild and have been reported to be associated with bacterial infections, which are major health hazard, as their excretion result in contamination of the environment leading to morbidity and mortality of other animals as well as significant economic losses for the zoo (Gopee *et al.*, 2000; Thachil *et al.*, 2010; Adesiyun *et al.*, 1998).

Since it was first identified in the early 1980s (Riley *et al.*, 1983). *Escherichia coli* (*E. coli*) is part of the normal microflora of animals and man. Most strains are harmless, but a limited number of serotypes are responsible for diarrhoea or more serious forms of illness. These strains are categorized as enteropathogenic, enterotoxigenic, enteroaggregative or enterohaemorrhagic according to their pathogenecity. Virulence is expressed in terms of their ability to adhere to or invade the mucosal surface of the intestine, and to produce haemolysins and toxins (Levine, 1987; Pohl, 1993). Enterohaemorrhagic E.coli (EHEC) are the most pathogenic strains among the verocytotoxin or Shiga toxin-producing E. coli (VTEC/STEC). They have been increasingly recognized as a cause of haemorrhagic colitis (HC) and the life-threatening haemolytic uremic syndrome (HUS) in man, particularly in children and the elderly (Karmali, 1989). Serotype O157:H7 and its non-motile variant O157:[H7] are the predominant cause of human infection (Boyce *et al.*, 1995). The pathogenecity of these important zoonotic pathogens is determined by the verotoxins VT1 and VT2, enterohaemolysin (Ehly) and the intimin adherence factor, an outer membrane protein encoded by the eaeA gene (Donnenberg *et al.*, 1993; Nataro and Kaper, 1998).

Recently, plasmid has been found to encode a series of important virulence enhancing properties of diverse pathogenic bacteria. In some instances, these plasmid-borne traits are essential to pathogenicity. Plasmid mediated toxin production has now been demonstrated for the heat-labile and heat-stable enterotoxins of *E. coli* (Freeman, 1985).

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Such bacterial pathogens have the ability to acquire multiple resistance genes (Ford *et al*., 2003) and cause major diseases as well as a number of minor diseases, the prevention of which depends largely on the efforts of medical, veterinary and agricultural bacteriologists (Singleton, 2004).

Considering the above rationale the present study was undertaken with the some distinct aims as mentioned below:

Isolation and identification of *E. coli* originating from faeces of zoo animal.

Pathogenecity studies of *E. coli* isolates of Zoo animals in mice and

Materials and Methods

The research work was conducted in the Department of Microbiology and Hygiene, Bangladesh Agricultural university (BAU), Mymensingh-2202 during the period of January 2012 to November 2012. A total of 62 fecal samples were collected from 7 different animals (Monkey, Bear, Lion, Deer, Baboon, Ass and Hayana) of two different Zoo (31 samples from each Zoo: 13 Monkey, 1 Bear, 1 Lion, 10 Deer, 4 Baboon, 1 Ass and 1 Hayana), Dhaka Zoo and Rajshahi Zoo. All the samples were cultured primarily in nutrient broth at 37ºC for 18-24 h, then subcultured onto the MacConkey, brilliant green and EMB agar by streak plate method (Cheesbrough, 1985) to observe the colony morphology (shape, size, surface texture, edge and elevation, colour, opacity etc). The organisms showing characteristic colony morphology of *E. coli* was repeatedly subcultured on to EMB agar until the pure culture with homogenous colonies were obtained. Gram's staining was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria. The organisms revealed gram negative, pink colored with rod shaped appearance and arranged in single or in pair were suspected as *E. coli.*

The motility test was performed by hanging drop technique as described by Cowan (1985) to differentiate the motile bacteria from the non-motile one. Hanging drop slide was prepared by broth culture and examined under 100X power objective. The motile organisms were suspected as *E. coli.*

Several biochemical tests were performed for confirmation of the culture. The biochemical tests were sugar fermentation tests, indole test, methyl red (MR) test, Voge's Proskauer (VP) test, catalase test etc. Pathogenicity of 50 representative *E. coli* isolates were determined by lethality assay in adult mice models.

Results and Discussion

A total of 62 samples, 31 from each Zoo (7 species of animals from such as Monkey-13, Deer-10, Bear-1, Baboon-4, Ass-1, Hyena-1, Lion-1) fecal samples were collected aseptically and subjected to primary isolation by propagating in nutrient broth followed by culture on different agar media.

E. coli was isolated successfully from 27 Samples out of 31 samples collected from seven species of animals of Rajshahi Zoo and 22 samples out of 31 samples collected from seven species of animals of Dhaka Zoo. All the *E. coli* isolates were able to produce smooth, circular, white to grayish white colonies on nutrient agar, bright pink colonies on MacConkey agar, Pinkish circular small colonies on Salmonella-Shigella (SS) agar, yellowish green colonies surrounded by an intense yellow green zone on BG agar and characteristic metallic sheen colonies on the EMB agar. Differences in colony morphology manifested by the isolates may be due to loosing or acquiring some properties by the transfer of host or choice of host tissue as observed by Dean (1990) and Dubreuil *et al.* (1991). In Gram's staining, the morphology of the isolated bacteria exhibited pink coloured, small rod shaped, Gram negative bacilli and in the hanging drop technique all the isolates revealed motility as observed by several authors (Buxton and Fraser, 1977; Freeman, 1985; Jones, 1987). In the present study almost all the isolates of *E. coli* fermented dextrose, maltose, lactose, sucrose and mannitol with the production of both acid and gas. also studied the biochemical characteristics of the different strains of *E. coli* isolated from different sources. They reported a little or no difference in these biochemical characters and stated that such similarity among the isolates might be due to presence of some common genetic materials. The results MR and indole test of the *E. coli* isolates were positive but V-P test and catalase test were negative which are in agreement with the reports of Buxton and Fraser (1977) and Honda *et al*. (1982).

Positive samples of *E. coli*

Table 1. Positive samples of *E. coli* in different Rajshahi Zoo animals

Name of Zoo	Nomenclature of Animal	Total number of samples	Positive samples for *E. coli*	Number of Pathogenic E. coli	Pathogenic E.coli%
Rajshahi Zoo	Monkey	13	11	0	0
	Bear	1	1	0	0
	Lion	1	1	0	0
	Deer	10	8	1	12.5%
	Baboon	4	4	1	25%
	Ass	1	1	1	100%
	Hayana	1	1	0	0

Table 2. Positive samples of *E. coli* in different Dhaka Zoo animals

Name of Zoo	Nomenclature of Animal	Total number of samples	Positive samples for *E. coli*	Number of Pathogenic E. coli	Pathogenic E. coli%
Dhaka Zoo	Monkey	13	10	0	0
	Lion	1	1	1	100%
	Deer	10	7	0	0
	Baboon	4	3	0	0
	Hayana	1	1	1	100%

In Rajshahi zoo among 31 animals fecal samples of animals 27 samples (Table 1)were positive for *E. coli* positive. Among seven types of animals all were *E. coli* positive making thereby a positive rate of 100%.

In Dhaka Zoo, Among 31 fecal samples of 7 type of animal species, 23 samples (Table 2) from five animals (except Ass

and Bear) were *E. coli* positive, which is not similar to that of Rajshahi.

Mice Lethality assay

Mice lethality assay was performed with the isolated *E. coli* strains in 6-8 weeks old mice by subcutaneous inoculation with 200 µl ($4x10^6$) in the abdomen.

Of the all mice inoculated with 50 *E. coli* isolates, five isolates of *E. coli* (three from Rajshahi zoo samples-4, 5, 6; 4-Deer, 5-Baboon, 6-Ass; and two from Dhaka Zoo samples-H, L; H-Hayana, L- Lion) caused lethal effect on mice showing blackish liver, congested lung, intestinal, mesenteric and subcutaneous haemorrhage.

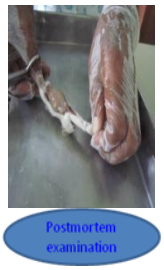


Fig. 1

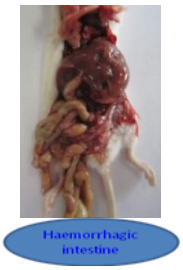


Fig. 2

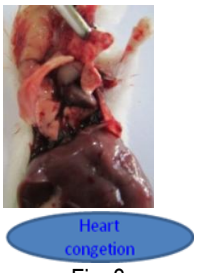
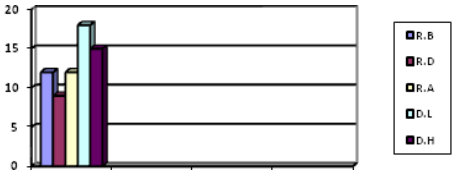


Fig. 3



Legend: R.B= Rajshahi Baboon, R.D= Rajshahi Deer, R.A= Rajshahi Ass, D.L= Dhaka Lion, D.H= Dhaka Hayana.

Fig 4. All Pathogenic E. coli died mice within 24 hour, that shows in the following graph.

Conclusions

In conclusion, our result showed that *E. coli* isolated from different animal fecal samples were found to be varied in virulence from high to avirulent and Mice lethality assay was assumed to be the best model for discriminating virulent and avirulent *E. coli*. Five *E. coli isolates* were pathogenic form among fifty E. coli isolates.

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