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Article

Isolation, identification and antibiogram profiles of enterovirulent *Escherichia coli* from diarrhoeic goat in some selected areas of Rangpur district of Bangladesh

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Abstract: Enterovirulent *Escherichia coli* remain as an important etiological agent of goat diarrhoea in Bangladesh. The present study was designed with a view to isolate and identifies *E. coli* from field cases. For this purpose, a total of 135 faecal samples (85 from diarrhoeic and 50 from apparently healthy goat) were collected during the period from January 2012 to July 2012 from different areas in Rangpur District. It was found that the prevalence of *E. coli* was higher (18.82 %) in diarrhoeic goats while it was lower (14.00 %) in non diarrhoeic goats. Age wise distribution of *E. coli* isolates were 26.42% in day old to 1 year, 10.53% in 1-2 years and 11.36% in above 2 years age of goat respectively. All the isolates of *E. coli* revealed greenish black colony with metallic sheen in Eosine methylene blue agar, bright pink color smooth transparent colony in MacConkey agar and slight pinkish smooth colony in Salmonella-Shigella agar. Gram stain and hanging drop techniques were performed with the cultured bacteria. Biochemical properties of the isolates were studied, and antibiotic sensitivity test was done by agar disk diffusion method. In Gram stain, the organisms revealed Gram negative, small rod shaped, occurs singly or paired. Biochemically, all of the isolates showed fermentation of dextrose, sucrose and maltose with the production of acid and gas, negative result to Voges-Proskauer test, positive result to Methylred test and differential result to Indol test. All the isolates of *E. coli* were highly sensitive to ciprofloxacin and gentamicin while moderately sensitive to colistin, livofloxacin and azithromycin and less sensitive to ceftraexon and tetracyclin and resistant to amoxycillin, ampicillin, erythromycin, and neomycin. Therefore, ciprofloxacin and gentamicin may be the antibiotics of first choice, and colistin, livofloxacin and azithromycin may be the second choice among the test antibiotics for the treatment of illness caused by these bacteria.

Keywords: *Escherichia coli*; diarrhoea; goat; antibiogram

1. Introduction

The major community health hazards both for men and animals in most countries of the world are diarrhoeal disease. It is resulted from the enteritis, which is the inflammation of the intestinal mucosa, characterized by abdominal pain, loose faeces, increase in faeces volume, faeces frequency, nausea or faeces fluidity that contain 70-95% water (dehydration). Several liter of fluid may be lost per day in severe cases of diarrhoea. The chronic form of diarrhoea may last for days or weeks and may culminate in death (Radostits *et al*., 1995). Diarrhoea of caprine occurs worldwide in goats of any age. Goat diarrhoea is responsible for poor growth in kids and a momentous loss of production both through morbidity and mortality. Some enteropathogens like bacteria, viruses, protozoa and helminths have been recognized to be associated with diarrhoea (Radostits *et al*., 1995). *Escherichia coli* (*E. coli*) is one of them.

E. coli a member of family Enterobacteriaceae is a short rod shaped, Gram negative, non-spore forming and usually peritrichous and fimbriate bacillus. A capsule or microcapsule is often present and a few strains produce profuse polysaccharide slime. *E. coli* was first isolated by Theobald Escherich in 1885 from faeces of infants. It serves as a major facultative anaerobe throughout its life as a harmless saprophyte but Larulle (1889) was the first to suggest the possible role of E. coli as a pathogenic organism. *E. coli* has been shown to be a normal inhabitant of the gastrointestinal tract of animal and man (Smith, 1965). The organism typically colonizes the infant gastrointestinal tract within hours of life and thereafter, both *E. coli* and the host derive mutual benefit (Drasar and Hill, 1974). In the debilitated or immunosupressed host or when gastrointestinal barriers are violated even normal non pathogenic strains of *E. coli* can cause infection. Enterovirulent or pathogenic *E. coli* is one of the most important groups of bacteria causing diarrhoea and extra intestinal infections in humans and animals (Levine, 1987). *Escherichia coli* is considered as the normal bowel flora of different species of mammals and birds but some strains of *E. coli* possess pathogenic character due to the acquisition of virulent factors. Microbial characteristics associated with virulent E. coli include production of enterotoxin, verotoxin, colicins and siderophores, type-1 pili and motility, resistance to the lytic action of the host complement and antibiotics (Dho and Lafont, 1984; Chulasiri and Suthienkul, 1989).

The enteric *E. coli* are divided into six groups on the basis of their virulence properties such as enterotoxigenic (ETEC, causative agent of diarrhoea in humans, pigs, sheeps, goats, cattle, dogs and horses), enteropathogenic (EPEC, causative agent of diarrhoea in humans, rabbits, dogs, cats and horses), enteroinvasive (EIEC, found only in humans), verotoxigenic (VTEC, found in pigs, cattle, dogs and cats), enterohaemorrhagic (EHEC, found in human, cattle, and goats) and enteroaggregative *E. coli* (EAggEC, found only in human). *E. coli* is a major pathogen of commercial poultry causing colibacillosis with manifestations such as airsacculitis, pericarditis, septicaemia, and death of the birds (about 28% deaths in Sonali birds) (Biswas *et al*., 2006). Enterotoxigenic *E. coli* (ETEC) is a major pathogen of animals, being responsible for diarrhoea in calves, lambs and goat kids resulting significant financial losses. Debnath *et al*. (1990) claimed 28% of the total death in calves occurred in first month of life and 50% of death during first week due to *E. coli* infection. It also causes on-farm contamination of different animal species (Fairbrother and Nadeau, 2006). ETEC is the most common cause of food and water-borne human diarrhoea worldwide. In developing countries, the incidence of enteric diseases due to ETEC is estimated about 650 million cases per year, resulting in 800,000 deaths, primarily in children of below five years old (Turner *et al*., 2006). So *E. coli* is an important zoonotic pathogen.

Goat rearing has become seriously impaired due to high mortality with diarrhoea like symptoms. The marginal and land-less farmers most easily live on rearing of goats in Bangladesh. So, goat is called the poor man's cow that is the second important livestock in Bangladesh which plays an important role in the rural economy and we can earn substantial amount of foreign currency by exporting skin and other by-products. Goats are very susceptible to *E. coli* infection. Two age groups appear to be susceptible, goats of 1-3 days of age and goats of 4-8 weeks old. Symptoms include diarrhoea, a rise in temperature, weakness and lack of appetite. This is soon followed by coma and death within a few hours. In older animals there is a tendency or infection to localize itself in the joints of survivors. Lesions include enlarged, haemorrhagic spleens, and the accumulation of synovial fluid and sometimes pus in affected joints (Blood *et al*., 1968). The *E. coli* infection is a disease of economic importance. The mortality rate due to E. coli infection in young age goat is higher of then older (Radostits *et al*., 1995). Due to *E. coli* infection in goat meat production is declined considerably. As a result the farmers who are economically dependent on goat rearing become looser.

Cultural characterization of *E. coli* by using different media and biochemical characterization by observing variable reaction to different sugars and chemicals are the basic rules for their identification. Zinnah, 2007 conducted an experiment on E. coli isolated from human, cattle, sheep, goat, chickens, duck, pigeon, drain sewages and soil. Antibiotics are widely used in case of diseased animal in the treatment of goat diarrhoea. In the context of Bangladesh, for many years antibiotic is randomly used for the treatment purpose. Knowledge of local antimicrobial therapy pattern is important in selecting the appropriate therapy. For the prevention and control of any microbial disease, prior isolation, identification and characterization of that particular etiological agent in a country is a precondition. The current study was undertaken to isolate and identify *E. coli* from the field cases and also to identify and selection of specific drugs to treat goat diarrhoea.

2. Materials and Methods

2.1. Sample collection

A total of 135 faecal samples were collected from different Veterinary hospitals in Rangpur District as shown in Table 1. Faeces were collected directly from rectum of goats with a history of suffering from diarrhoea and also apparently healthy one. About 05-10 grams of faeces were collected from each animal by using clean plastic

gloves and kept in a sterilized screw caped vial. All the samples were collected aseptically from the rectum by using finger or directly from fallen faeces. The samples were carried to the Bacteriology Laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh in an ice box contained ice and processed for the isolation and characterization of bacteria subsequently. And the remaining samples were stored at 4°C for further use.

2.2. Laboratory preparation

All items of glass wares including test tubes, pipettes, cylinder, flasks, conical flasks, glass plate, slides, vials soaked in a household dishwashing detergent solution ('Trix' Recket and Colman Bangladesh Ltd.) for overnight, contaminated glassware were disinfected in 2% sodium hypochloride solution prior to cleaning. The glassware were then cleaned by brushing, washed thoroughly and finally sterilized either by dry heat at 160^0C for 2 hours or by autoclaving for 15 minutes at 121^0C under 15 lbs pressure per square inch. Autoclaved items were dried in a hot air oven over at 50^0C. Disposable plastic were (micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50^0C for future use.

2.3. Bacteriological examination of feacal sample

1% Sterile peptone water was added to all feacal samples and mixed to give a semi-solid consistency and bacterial culture was done for individual pathogens as described by Merchant and Packer (1976), OIE (2000) and Carter (1991). The samples were first cultured into the nonselective enriched media such as nutrient broth (NB) and nutrient agar (NA) media and incubated at 37^0C for 24 to 48 hrs for the growth of bacteria. Then the samples were subcultured into the different selective media and incubated at 37^0C for 24 to 48 hrs for identification of bacteria by their morphological characteristics including size, shape, surface texture, edge, elevation, color, opacity etc. were studied as described by Merchant and Packer (1976). The media used were MacConkey (MC) agar, Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar, Mueller Hinton (MH) agar, Triple sugar iron (TSI) agar, Basic sugar media (dextrose, lactose, mannitol and maltose), Motility Indole Urease (MIU) agar base media, Methyle Red-Voges Proskauer medium base. Several biochemical tests such as Indole test, MIU test, Methyl red (MR) test, Voges-Proskauer (VP) test, Triple Sugar Iron test and Carbohydrate fermentation test were performed according to Holt *et al*. (1994) to identify the biochemical characteristics of the bacterial isolates.

2.3.1. Bacterial motility test

The motility test was performed to differentiate motile bacteria from the non-motile one. Before performing the test, a pure culture of the organism was allowed to grow in Nutrient broth. One drop of cultured broth was placed on the coverslip and was placed invertedly over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the coverslip to prevent air current and evaporation of the fluid. The hanging drop slide was then examined carefully under 100 power objective of a compound microscope using immersion oil. Movement of organism under microscope was observed due to presence of flagella/fimbri (Cowan, 1985).

2.3.2. Determination of morphology of bacteria by Gram's staining method

The Gram's staining method was performed as described by Merchant and Packer (1976). Briefly, a small colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violet solution was then applied on the smear to stain for two minutes and then washed with running water. Gram's iodine was added to act as mordent for one minutes and then again washed with running water. Acetone alcohol was then added, which act as a decolorizer. After 10 second washing with water and safranine was added as counter stain and allowed to stain for 1 to 2 minutes. Then the slide was washed with water, blotted and dried in air and then examined under microscope with 100 × objectives.

2.4. Antimicrobial susceptibility test

The antimicrobial susceptibility test for *E. coli* was performed on freshly prepared Mueller Hinton agar (HiMedia, India) by disc diffusion method, as described by Khan *et al*. (2005). Ten different antibacterial disc (CARTIDGE DISC), manufactured by MAST DIAGNOSTICS, Merseyside, UK were selected, viz- Amoxicillin (25 µg/disc), Ampicillin (10 µg/disc), Levofloxacine (5 µg/disc), Ciprofloxacin (5 µg/disc), Ceftraexon (30 µg/disc), Cloxacillin (5 µg/disc), Gentamicin (10 µg/disc), Tetracycline (30 µg/disc), Neomycin (30 µg/disc), Colistin sulphate (25 µg/disc), Azithromycin (15 µg/disc) and Erythromycin (15 µg/disc) . The

isolated bacteria were analyzed for antimicrobial susceptibility test were expressed as either resistant or sensitive, as per the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012).

3. Results and Discussion

Among bacterial pathogens worldwide, enterovirulent *Escherichia coli* ranks among the most common causative agents of bacterial diarrhoea in several animal species as well as in humans (Turkyilmaz *et al.*, 2013). The fecal carriage of *E. coli* in goats has been considered as a prime source of fecal contamination of food and water (Bist *et al*., 2014). Species of *E. coli* were isolated from feacal samples of goats on the basis of morphological, cultural and biochemical characteristics. The detailed results are shown in Table 2. *E. coli* form smooth, glistening and opalescent colonies on NA, greenish black colony with metallic sheen on EMB agar, rose pink lactose fermenter colony on MacConkey agar and slight pinkish color smooth colony on SS agar. *E. coli* showed Gram-negative (pink) rods, arranged in single, paired or in short chain, short plump rods in Gram's Method. Carbohydrate fermentation test showed that *E. coli* fermented glucose, maltose and lactose producing both acid and gas. Methyl Red (MR) test, Indole test and Motility test were positive, whereas Voges–Proskauer (VP) and Urease test were negative. These findings were similar to other studies conducted by Beutin *et al.* (1993), McKee *et al.* (1995), Abdullah *et al.* (2010) and Islam *et al.* (2017).

A total of 135 feacal samples consist of 85 diarrhoeic and 50 non diarrhoeic of goat were collected during the study period. Prevalence of *E .coli* found as 18.82% (n=16/85) in diarrohoeic and 14% (n=7/50) in nondiarroheic goat respectively (Table 3). The overall prevalence of *E. coli* found as 17.04% (n=23/135) in faecal samples of goat (Table 3). This rate was corroborate with Bhat *et al*. (2008) and Turkyilmaz *et al.* (2013) who reported 17.8%, Ashraf (2016) who reported 14% faecal samples positive for *E. coli.* Wani *et al*. (2003) reported 6% was positive for *E. coli* lower than our findings while Abdullah *et al*. (2010) and Adefarakan *et al*. (2014) reported 41.67% and 42.2% faecal samples from goats, positive for *E. coli*, higher than our findings. However, this variation in the prevalence might be due to sampling pattern, socio-demographic condition, climatic and geographical diversity of the goats examined. The prevalence of *E. coli* isolates found in different hospitals as 15.38% (DVHR), 21.43% (UVHRS), 20% (VHKTR) and 15.79% (VHGTR) in diarrhoeic goat respectively (Table 4) while 10% (DVHR), 17.65% (UVHRS), 16.67% (VHKTR) and 9.09% (VHGTR) in non diarrhoeic goat respectively (Table 4). The overall prevalence of *E. coli* isolates found in different hospitals as 13.04% (DVHR), 20% (UVHRS), 18.92% (VHKTR) and 13.33% (VHGTR) respectively. Table 5 revealed age wise distribution of *E. coli* isolates were 26.42% in day old to 1 year, 10.53% in 1-2 years and 11.36% in above 2 years age of goat respectively. The prevalence of *E. coli* infection in kids was higher compare to young and adult. The reasons behind higher rate in kids might be due to lack of proper acquired immunity, susceptibility to various *E. coli* pathotypes, nutritional insufficiency, lack of bio-security and hygienic condition in the farms (Zaki *et al*., 2010; Jafari *et al*., 2012). *E. coli* has been reported as the important causative agent of diarrhoea that causes great economic losses in the farming industry (Gokce *et al*., 2010). Goats have been identified as major reservoirs which cause asymptomatic infections in animals and which can pass through the food chain to cause clinical disease in man (Arshad *et al*., 2006; Kiranmayi *et al*., 2010). *E. coli* infection is particularly a challenge for the rural communities who live in close proximity of goats, and have no or least knowledge about pathogenicity of bacteria and the transmission of disease. Public Health awareness including safe and hygienic practices, are of prime importance in decreasing the occurrence of *E. coli* infection and its spread to humans especially to the individuals closely associated with rearing and management of goats.

Table 1. Number of fecal samples collected from different areas of Rangpur District.

Name of veterinary hospital	Total number of samples collected	
	Diarrhoeic samples	Non diarrhoeic samples
DVHR	13	10
UVHRS	28	17
VHKTR	25	12
VHGTR	19	11
Total	85	50
Grand total	135	

Legends: DVHR = District Veterinary hospital, Rangpur; UVHRS = Upazila Veterinary Hospital, Rangpur Sadar; VHKTR = Veterinary Hospital Kaunia Thana, Rangpur; VHGTR = Veterinary Hospital Gangachara Thana, Rangpur.

Table 2. Cultural, morphological and biochemical properties of *E. coli* from fecal sample.

	Test parameter	Observation
Culture media	Nutrient broth	Turbidity of the media
	Nutrient agar	Smooth, glistening and opalescent colony
	EMB (Eosin methylene blue) agar	Greenish black colony with metallic sheen
	MacConkey agar	Rose pink lactose fermenter colony
	SS (Salmonella-Shigella) agar	Slight pinkish smooth colony
Morphology	GN, pink color, single or paired, small rod shaped	
Biochemical tests		
TSI	Butt & Slant yellow color	Glucose, Maltose, Lactose all are fermented
	Gas bubble throughout the media	+
	Black colour (H2S production)	-
MR-VP	MR- Red color	+
	VP-No color change	-
MIU	M-Turbid whole medium	Motile
	I-Pink color neck of the medium	+
	U-No color change whole medium	-

Legends: TSI = Triple Sugar Iron; MR= Methyle Red; VP=Voges Prauskeur; MIU=Motility Indole Urease

Table 3. Prevalence of E. coli Isolates from diarrhoeic and non diarrhoeic goat.

Samples from condition of goat	No. of sample examined	No. of *E. coli* positive samples	Percentage of *E. coli* positive samples
Diarrohoeic	85	16	18.82 %
Non diarrohoeic	50	07	14.00 %
Total	135	23	17.04 %

Table 4. *E. coli* isolates from diarrhoeic and non diarrhoeic goat in different Veterinary hospitals in Rangpur district.

Location	DVHR		UVHRS		VHKTR		VHGTR	
Sample source	Diarrhoeic goat	Non diarrhoeic goat	Diarrhoeic goat	Non diarrhoeic goat	Diarrhoeic goat	Non diarrhoeic goat	Diarrhoeic goat	Non diarrhoeic goat
No. of the sample examined	13	10	28	17	25	12	19	11
No. & % positive of *E. coli*	02 (15.38)	01 (10.00)	06 (21.43)	03 (17.65)	05 (20.00)	02 (16.67)	03 (15.79)	01 (09.09)
No. & % Total distribution of *E. coli*	03(13.04 %)		09(20.00 %)		07(18.92 %)		04(13.33 %)	

Legends: DVHR = District Veterinary hospital, Rangpur; UVHRS = Upazila Veterinary Hospital, Rangpur Sadar; VHKTR = Veterinary Hospital Kaunia Thana, Rangpur; VHGTR = Veterinary Hospital Gangachara Thana, Rangpur.

Table 5. Age wise distribution of *E. coli* isolates.

Age of goats	No. of sample examined	No. of *E. coli* positive samples	Percentage of *E. coli* positive samples
Day old-1 year	53	14	26.42%
01-2 years	38	04	10.53%
>2 years	44	05	11.36%

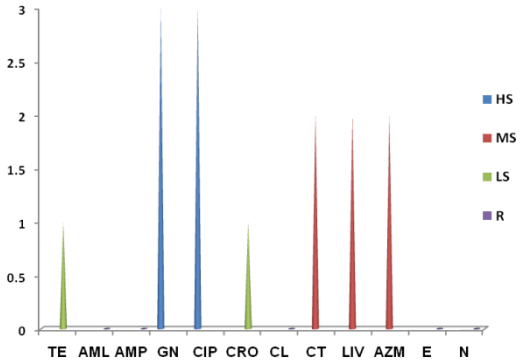

Figure 1. Antibiogram profile of *E. coli* against tetracycline (TE), amoxicillin (AML), ampiciline (AMP), gentamicin (GN), ciprofloxacin (CIP), ceftraexon (CRO), Cloxacillin(CL), Colistin (CT), levofloxacin (LIV), Azithromycin(AZM), Erythromycin (E) and neomycin (N)

Antibiogram means the test that is done in laboratory or in-vitro condition to detect the sensitivity of antibiotics against certain bacteria which is responsible for specific diseases. On the other hand, this test is done to measure the ability of antibiotics to prevent the growth of bacteria in in-vitro condition or in a suitable or in a suitable environment outside the host body due to the following importance this test was done. Antibiotic sensitivity test was performed by Agar disc diffusion methods. The present study revealed that the *E. coli* isolates of goat were highly sensitive to ciprofloxacin and gentamicin while moderately sensitive to colistin, livofloxacin and azithromycin and less sensitive to ceftraexon and tetracyclin (Figure 1). The isolates of goat *E. coli* were resistant to amoxycillin, ampicillin, cloxacillin, erythromycin, and neomycin (Figure 1). It has been reported that isolates of *E. coli* from humans and animals express high resistance to common antibiotics (Kang *et al*., 2005). Adefarakan *et al.* (2014) reported *E. coli* was frequently resistant to amoxicillin while Rashid *et al.* (2006) reported *E. coli* was frequently resistant ampicillin and highly sensitive against chloramphenicol, ciprofloxacin, norfloxacin and gentamycin. Islam *et al.* (2016) found *E. coli* isolates were high level of resistant against ampicillin and amoxicillin-clavulanic acid and highly sensitive to ceftriaxone, cefotaxime and gentamicin. The findings of our study was also similar to Radwan *et al.* (2014) who reported that *E. coli* was resistance to ampicillin and amoxiclillin. Begum *et al*. (2015) who reported *E. coli* was highly sensitive to ciprofloxacin followed by gentamicin and tetracycline. Studies on antimicrobial susceptibility of *E. coli* from different animal species showed an increase in the incidence of resistance over the years as a result of the wide-spread use of antibiotics in animals (Cid *et al*., 1996). However prescription of antimicrobials precedes the antimicrobial sensitivity test and antibiotic control strategy should be regulated to prevent the emergence of the drug resistance.

4. Conclusions

Goats are potential and economic livestock of Bangladesh. A large number of goat populations are decreasing due to diarrhoea caused by *Escherichia coli* in every year. The presence of *E. coli* in animal faeces provides the potential for these organisms to enter the food chain via faecal contamination, from animal to human contact, both direct and indirect, human-to-human contact. Goat meat can transmit infections and diseases either through handling during preparational procedures or as a result of ingestion by the consumer. The incidence of multiple antibiotic resistance in *E. coli* isolated from apparently healthy goats is high in the study area continues to be the challenge for the people residing in the rural areas where the peoples live in close proximity of goats have least knowledge about the pathogenecity of the bacteria. Hence, indiscriminate use of antibiotics in the animal husbandry sector should be discouraged.

Conflict of interest

None to declare.

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