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MOLECULAR IDENTIFICATION AND ANTIBIOGRAM PROFILES OF *ESCHERICHIA COLI* ISOLATED FROM APPARENTLY HEALTHY AND DIARRHEIC GOATS

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ABSTRACT

The present study was designed for the cultural, biochemical characterization and molecular detection of *E. coli* from apparently healthy and diarrheic goats in and around BAU campus including their antibiogram study. A total of 50 fecal samples were collected among which 13 originated from diarrheic goat and 37 from apparently healthy goats. Out of 50 samples, 35 were found positive for *E. coli i.e.,* overall 70% occurrence. Occurrences of *E. coli* from diarrheic and apparently healthy goats were 92% and 62% respectively. Occurrences were 60%, 80% and 70% in case of BAU Goat Farm, Veterinary Teaching Hospital and Boyra respectively. On age basis 93%, 54%, 66% and 54% samples originated from 6 months, 7-12 months, 13-18 months and 19 months aged goats were found positive respectively. Occurrences of *E. coli* on the basis of sex were 78% for male and 62% for female. In case of breed, the occurrences were 69% in Black Bengal and 100% in for Jamunapari. Molecular detection was done by PCR and 13 out of 20 isolates tested gave the bands at the 585 bp specific for *E. coli* 16S rRNA gene. All the isolates (100%) were found sensitive to ciprofloxacin and norfloxacin; 100% and 35% were intermediately resistant to tetracycline and gentamicin respectively and 25% isolates were resistant to streptomycin. Ciprofloxacin and norfloxacin were found to be the best choice of antibiotics for the treatment of colibacillosis in goats in the study area.

Key words: *E. coli*, goats, occurrence, PCR, antibiogram profile

INTRODUCTION

Livestock population in Bangladesh is currently estimated to comprise 25.7 million cattle, 0.83 million buffaloes, 14.8 million goats, 1.9 million sheep, 118.7 million chicken and 34.1 million ducks (Banglapedia, 2015). Among livestock population goats are known as poor person's bank or poor family's insurance policy and distributed mainly with landless and marginal farmers, but now-a-days the commercial goat farming is also gaining much importance.

The major loss faced by the goat farmers is in terms of mortality and morbidity of young animals and is mainly due to diarrhea which is a complex interaction of etiological, immunological, and managemental factors. *Escherichia coli* play an important role in causing diarrhea and other infectious diseases in goats. Pathogenicity of *E. coli* strains are due to the presence of one or more virulence factors including invasiveness factors like invasins, heat labile, heat stable enterotoxins, verotoxins and colonization factors or adhesins (Kaper *et al*., 2004). Pathogenic *E. coli* are divided into two types- Enteropathogenic *E. coli* and Uropathogenic *E. coli*. Enteropathogenic *E. coil* is further grouped into Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAggEC), Enterohemorrhagic *E. coli* (EHEC) which are responsible for causing diarrhea or related illness (Islam *et al.*, 2016).

In case of diarrhea in goat or other animals, the precise role of *E. coli* is not clear because this organism is part of normal intestinal flora in both healthy and diseased animals. At least three explanations for the presence of *E. coli* in the gut of diarrheic animals are possible: (a) *E. coli* isolate may be a part of normal intestinal flora and the diarrhea has another etiology. (b) The mechanism responsible for infection creates an imbalance in the digestive system that enables *E. coli* to grow and perhaps to secondary diarrhea. (c) *E. coli* alone is responsible for causing diarrhea (Radostits *et al*., 2000). The economic aspect of diarrheal diseases in goats and their mortality and morbidity is a matter of great concern to the livestock owners. It can be controlled following the maintenance of strict hygienic and sanitary measures in addition to antibiotic therapy.

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Antibiotics are widely used for the treatment of diarrhea in goats. Incomplete course of treatment and continuous indiscriminate use of antibacterial drugs against diarrheal infection of man and animal might have influenced to produce a new generation of virulent and resistant type of bacteria (Marshall *et al*., 1990). *Escherichia coli* has become resistant to many antimicrobials through the acquisition of mobile drug resistance genes and the incidence of multiple antibiotic resistant *E. coli* strains has been increasing. Different parameters including the isolation, identification, vaccination, plasmid profiling, antibiotic sensitivity and epidemiological investigation of *E. coli* of different species were studied in Bangladesh by Islam *et al.* (2008), Nazir *et al.* (2005); Abdullah *et al.* (2010), Islam *et al.* (2016) and Gupta *et al*. (2016). However, as far as we know molecular detection and antibiogram study of *E. coli* from healthy and diarrheic goats in BAU and surrounding areas recently were not systematically focused. Therefore, the present study was performed to isolate and molecular detection of *E. coli* inhabiting in feces of goats and to assess antibiogram profiles of the isolated *E. coli.*

MATERIALS AND METHODS

This research work was conducted during the period of January, 2016 to May, 2016 in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh.

Collection of samples

A total of 50 rectal swab samples were aseptically collected from the diarrhoeic goats of BAU, Mymensingh and surrounding area. Among 50 samples, 20 originated from BAU Goat Farm (19 apparently healthy and one 1 diarrheic goat), 20 from Veterinary Teaching Hospital, BAU (10 apparently healthy and 10 diarrheic goats) and 10 from Boyra, Mymensingh (8 apparently healthy and 2 diarrheic goats). After collection, the samples were transported to the Bacteriology, Department of Microbiology and Hygiene, BAU as soon as possible for the bacteriological examinations and further characterization.

Isolation of *E. coli*

The isolation and identification of *E. coli* were performed according to the method described by Cowan (1985).The isolated bacteria was initially identified as *E. coli* by observing their cultural characteristics, morphology by Gram's stain, biochemical tests, motility test and finally confirmed by PCR through amplification of *E. coli* specific 16S RNA gene.

Molecular detection of *E. coli* by PCR targeting *E. coli* 16S rRNA gene

Extraction of DNA from the *E. coli* was carried out by conventional boiling and rapid cooling method (Medici *et al*., 2003). In brief 200 µl deionized water was taken into an eppendorf tube, a pure colony of *E. coli* from nutrient agar was mixed with the deionized water. The tube was then transferred to boiling water for 10 minutes then it was immediately taken to the icebox for cold shock about 10 minutes. After cold shock centrifugation at 10,000 rpm for 10 minutes was done. The supernatant was collected which was used as template DNA. Details of the oligonucleotide primers used for the amplification of 16S rRNA gene of *E. coli* as shown in Table 1. The PCR reaction mixture (25µl) for *E. coli* was prepared using 12.5 µl master mixture (Promega, USA), 10 pmol primer (Bioneer, South Korea) of each, 5µl DNA template and 5.5 µl nuclease free water. The cycling conditions consisted of initial denaturation for 5 minutes at 95°C, followed by 30 cycles of denaturation at 94°C for 0.5 minutes, annealing at 58°C for 1 minute and extension at 72°C for 1 minute. The final extension was conducted at 72°C for 10 minutes. Amplification was performed in a thermal cycler (Astec, Japan). The amplified products were electrophoresed into 1.5% agarose (Sigma-Aldrich, USA) gel at 80 volt for 45 minutes and then visualization was done under Gel doc/UV trans-illuminator (BioRad).100 bp DNA size marker (Promega, USA) was used.

Table 1. Primer sequence, target gene and predicted size of amplified product of *E. coli* specific 16S rRNA

Primer name	Sequence (5'-3')	Target gene	Amplicon size (bp)	Reference
ECO-1	GACCATCGGTTTAGTTCACAGA	*E. coli* 16S rRNA	585	Schippa *et al.*(2010)
ECO-2	CACACGCTGACGCTGACCA			

Antibiogram Study

Antibiogram study was performed by employing the Kirby-Bauer disc diffusion method (Bauer *et al*., 1959) using five different commercially available antibiotic discs (HiMedia, India and Oxoid Ltd., England) on Mueller-Hinton agar (HiMedia, India) to assess the susceptibility and resistance pattern of the *E. coli* isolates. The selected antibiotics used were ciprofloxacin (5 µg/disc), gentamicin (10 µg/disc), norfloxacin (10 µg/disc), streptomycin (10 µg/disc), and tetracycline (30 µg/disc).The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2012). In this study, multidrug resistant *E. coli* was identified by considering resistant to 2 or more drugs.

RESULTS AND DISCUSSION

Out of 50 rectal swab samples 35 were found positive for *E. coli*. The Gram negative small rod shaped organisms produced circular, smooth, colorless colony on NA. All the isolates produced greenish-black colonies with metallic sheen on EMB agar and bright pink or red colored colonies on MC agar as described by Merchant and Packer (1967). All the *E. coli* isolates were found positive to motility test and fermented the five basic sugars with the production of acid and gas. Among the 35 *E. coli* 20 were selected for molecular detection by PCR targeting 16S rRNA gene of *E. coli* (amplicon size 585 bp). Among them 13 isolates were found *E. coli* positive by PCR.

The occurrence of *E. coli* (Table 2) from diarrheic goats was 92% (n= 23/37) and apparently healthy goats was 62% (n=12/13). Islam *et al.* (2016) also observed higher rate of presence of *E. coli* in diarrhoeic goat than healthy goat. In BAU goat farm the prevalence of *E. coli* was 60% (n=12/20), 80% (n=16/20) in Veterinary teaching hospital and 70% (n=7/10) in Boyra. The prevalence of *E. coli* was 93% (n=14/15) in 6 months aged goats, 54% (n=7/12) in 7-12 months aged goats, 66% (n=8/11) in 13-18 months aged goats and 54% (n=6/12) in 19 months aged goats. In male goats the prevalence of *E. coli* was 78% (n=18/23) and 62% (n=17/27) in female goats. In case of breed the prevalence of *E. coli* was 69% (n=34/49) in Black Bengal goats and 100% (n=1/1) in Jamunapari goat. Islam *et al.* (2016) detected 52% fecal sample positive for *E. coli* in goat rectal swab in Cox's Bazar area. Among the isolated *E. coli* many could be pathogenic. Islam *et al.* (2008) detected occurrence of 10% STEC in rectal swab of goat collected from slaughter house in Bangladesh. These observed variations in the occurrence of *E. coli* in different categories of goat population in our study could be due to the different hygiene and management practice of the goat owners.

Table 2. Occurrence of *E. coli* on the basis of different parameters

parameter		No. of samples analyzed	No. of samples found positive for *E. coli*	Prevalence (%)
Health status	Apparently Healthy	37	23	62
	Diarrheic	13	12	92
	Total	50	35	70
Location	BAU Goat farm	20	12	60
	VTH	20	16	80
	Boyra	10	7	70
	Total	50	35	70
Age	≤6 months	15	14	93
	7-12 months	12	7	58
	13-18 months	11	8	66
	≥19 months	12	6	54
	Total	50	35	70
Sex	Male	23	18	78
	Female	27	17	62
	Total	50	35	70
Breed	Black Bengal	49	34	69
	Jamunapuri	1	1	100
	Total	50	35	70

*VTH= Veterinary Teaching Hospital

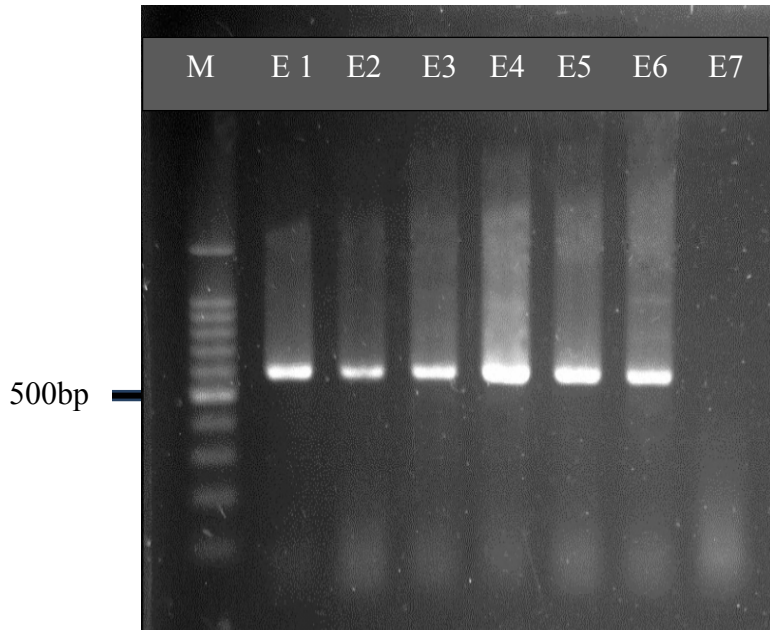

Figure 1. PCR for *E. coli* 16S rRNA gene. Lane M: 100 bp DNA Marker; Lanes E1-E5 isolated *E. coli*, E6 positive control and E7 negative control. Expected amplicon size is 585 bp.

A total of 20 randomly selected isolates were subjected to the antibiogram study. The results of antibiogram study are presented in Table 3 and 4. Among them 10 isolates were taken from apparently healthy goats and 10 were taken from diarrheic goats. On the basis of health status, isolated *E. coli* both from apparently healthy and diarrheic goats were found 100% sensitive to ciprofloxacin and norfloxacin; 20% were resistant to tetracycline and 80% were intermediately resistant to tetracycline. From apparently healthy goats 30% isolates were found intermediately resistant to gentamicin and 40% isolates were resistant to streptomycin. From diarrheic goats 40% were found intermediately resistant to gentamicin and 10% isolates were resistant to streptomycin. Overall, 100% isolates were found sensitive to ciprofloxacin and norfloxacin; 90% and 35% were intermediately resistant to tetracycline and gentamicin respectively; 25% isolates were resistant to streptomycin and 2% isolates were resistant to tetracycline. In this study, *E. coli* isolated from goats were found highly sensitive to ciprofloxacin. Similar type of antibiogram profile of *E. coli* originating from various sources has earlier been reported by Nazir *et al*. (2005). Among the *E. coli* studied, 2 isolates were found multidrug resistant *i.e.,* resistant to streptomycin and tetracycline. Islam *et al.* (2016) also reported the occurrence of multidrug resistant *E. coli* in goat population in Bangladesh.

Table 3. Antibiogram profiles of the isolated *E. coli* from apparently healthy and diarrheic goats

Sources of *E. coli* Isolates	Resistant		Intermediate		Sensitive	
	Antibiotic	No. (%)	Antibiotic	N0. (%)	Antibiotic	No. (%)
Apparently Healthy (n=10)	S	4(40%)	S	0(0%)	S	6(60%)
	GEN	0(0%)	GEN	3(30%)	GEN	7(70%)
	CIP	0(0%)	CIP	0(0%)	CIP	10((100%)
	NOR	0(0%)	NOR	0(0%)	NOR	10(100%)
	TE	0(0%)	TE	10(100%)	TE	0(0%)
Diarrheic (n=10)	S	1(10%)	S	0(0%)	S	9(90%)
	GEN	0(0%)	GEN	4(40%)	GEN	6(60%)
	CIP	0(0%)	CIP	0(0%)	CIP	10(100%)
	NOR	0(0%)	NOR	0(0%)	NOR	10(100%)
	TE	2(20%)	TE	8(80%)	TE	0(0%)

*n=No. of isolates subjected to antibiotic profile, S=Streptomycin, GEN= Gentamicin, CIP=Ciprofloxacin, NOR= Norfloxacin, TE= Tetracycline.

Table 4. Overall percentages (%) of antibiogram profiles of isolated *E. coli* from collected samples

Antibiotics	Resistant	Intermediate	Sensitive
Streptomycin	5(25%)	0(0%)	15(75%)
Gentamycin	0(0%)	7(35%)	13(65%)
Ciprofloxacin	0(0%)	0(0%)	20(100%)
Norfloxacin	0(0%)	0(0%)	20(100%)
Tetracycline	2(10%)	18(90%)	0(0%)

The findings of this research work would certainly help the veterinary practitioners to select the proper antibiotics against diarrhoea in goats of Bangladesh, particularly in the study area. If the prescribers prescribe suitable antibiotics against diarrhoea in goats then it would be possible to overcome the multi-drug resistant problem of bacteria, otherwise successful antibiotic therapy against diarrhoea in goats would not be possible.

CONCLUSIONS

E. coli were successfully isolated from healthy and diarrheic goat and confirmed by PCR. Overall occurrence of *E. coli* was 70%. Based on the present study, it may be concluded that use of ciprofloxacin and norfloxacin will be of first choice of treatment against *E. coli* infection in goats located at the study area. Findings of this study have suggested that multidrug resistant *E. coli* isolated from goat might be an important concern for veterinary practitioners. Nevertheless, more studies are needed to clearly understand the genomic diversity in *E. coli* as well as molecular mechanisms of virulence and development of antimicrobial resistance.

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REFERENCES

1. Abdullah ASM, Khan MSR, Alam M, Haq F and Hassan J (2010). Isolation and Molecular Characterization of *Escherichia coli* from goat of apparently healthy and clinical cases. *Bangladesh Journal of Microbiology* 27: 14-17.
2. Banglapedia (2015). Livestock animal of Bangladesh. http://en.banglapedia.org/index.php?title=Livestock.
3. Bauer A, Kirby W, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45: 493.
4. CLSI (2012). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute.
5. Cowan ST (1985). Cowan and Steel's Manual for Identification of Medical Bacteria. 2nd edn., Cambridge University Press, Cambridge, London. pp. 138-139.
6. Gupta MD, DAS A, Islam MZ and Biswas PK (2016). Prevalence of sorbitol non-fermenting Shiga toxin-producing *Escherichia coli* in Black Bengal goats on smallholdings. *Epidemiology and Infection* 8: 1-8.
7. Islam K, Ahad A, Barua M, Islam A, Chakma S, Dorji C, Uddin MA, Islam A and Ahasan ASML (2016). Isolation and epidemiology of multidrug resistant *Escherichia coli* from goats in Cox's Bazar, Bangladesh. *Journal of Advanced Veterinary and Animal Research* 3:166-172.
8. Islam MS, Mondol AS, Boer E, Beumer RR, Zwietering MH, Talukder KA and Heuvelink AE (2008). Prevalence and genetic characterization of shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. *Applied and Environmental Microbiology* 74: 175414-5421.
9. Marshall B, Petrowski D and Levy SB (1990). Inter and intra species spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage. National Academy, USA. 87: 6609-6613.
10. Medici D, Croci L, Delibato E, Pasquale SD, Filetici E and Toti L (2003). Evaluation of DNA extraction methods for use in combination with SYBR Green I Real-Time PCR to detect *Salmonella enterica* serotype *enteritidis* in poultry. *Applied and Environmental Microbiology* 69: 3456-3461.
11. Nazir KHMNH, Rahman MB, Nasiruddin KM, Akhtar F, Khan MFR and Islam MS (2005). Antibiotic sensitivity of *Escherichia coli* isolated from water and its relation with plasmid profile analysis. *Pakistan Journal of Biological Sciences* 8: 1610-1613.
12. Radostits OM, Gay CC, Blood DC and Hinchcliff KW (2000).Veterinary Medicine: A Textbook of Disease of Cattle, Sheep, Pigs, Goats and Horses. 6th edn.,WB Saunders, New York, USA. pp. 467.
13. Schippa S, Iebba V, Barbato M, Nardo GD, Totino V, Checchi MP, Longhi C, Maiella G, Cucchiara S and Conte MP (2010). A distinctive microbial signature in celiac pediatric patients. *BMC Microbiology* 10: 175-185.