

Original Article

Isolation and epidemiology of multidrug resistant *Escherichia coli* from goats in Cox's Bazar, Bangladesh

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ABSTRACT

Objective: The investigation was undertaken to measure the epidemiological features and antimicrobial sensitivity patterns of *Escherichia coli* among different breeds of goats in Cox's Bazar, Bangladesh.

Materials and methods: A total of 150 rectal swab samples were collected from selected goats. The samples were subjected for the isolation of *E. coli* through a series of conventional bacteriological and biochemical techniques. The isolated *E. coli* were used for assessing antimicrobial susceptibility by disk diffusion method. Besides prevalence study, nine risk factors were studied; the risk factors were breed, age, hygienic status, sex, history of recent transportation, season, diarrhea, body condition score, and source of drinking water.

Results: The overall prevalence of *E. coli* in the rectal swabs of goats was 52% (n=78/150). The young goats were highly susceptible (65.0%; n=52/80) to *E. coli* infection as compared to adults (37.1%; n=26/70). The prevalence was higher (66.7%; n=42/63) in the goats that was reared in poor hygienic condition as compared to the goats that were reared under good hygienic condition. The influence of the source of drinking water on *E. coli* prevalence was found to be higher both in pond (63.6%; n=7/11) and municipality (61.4%; n=51/83) supplied water as compared to tube-well (35.7%; n=20/56). The goats having recent transportation history showed higher prevalence (64.8%; n=35/54). The prevalence was higher in diarrheic goats (62.0%; n=49/79) than non-diarrheic goats. Among the *E. coli* isolates (n=78), 31 were found to be multidrug resistant (MDR) to 3 to 8 subclasses of antimicrobials.

Conclusion: Presence of MDR *E. coli* in the studied goats suggest the probable acquisition, development and transmission of MDR *E. coli* through a number of influencing factors to other animals and potentially to human.

KEYWORDS

Antibiogram, *Escherichia coli*, Epidemiology, One Health, Risk factors

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INTRODUCTION

Goat farming is one of the important components of the livestock industry in Bangladesh, where it is reared mainly as a source of meat, good quality skin, and sometimes for milk. Farmers rear goats mostly as a subordinate occupation to supplement their livelihood. Goat farming is not only a commercial enterprise but also a mode of life which contributes substantially in farmer's income as well as in national economy. According to Food and Agricultural Organization (FAO), meat and skin of goats contribute 38% and 28% of the whole livestock meat and skin production in Bangladesh ([Sarker and Islam, 2011](#)).

Among notable diseases of goat, diarrhea is considered as common gut associated illness throughout the world. A number of research has been undertaken worldwide to determine pathogenic bacterial burden which may be an issue of concern for public or human health ([Bist et al., 2014](#)). Though *E. coli* is considered as an intestinal microflora of warm blooded animals and humans, some *E. coli* could be emerged different stratagems for generation of infection in the host and cause crippling and fatal diseases in human and animals ([Bélanger et al., 2011](#)).

The gastrointestinal tract (GIT) of endotherms is a chamber to a number of *E. coli* pathotypes including enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC) and diffusely adherent (DAEC) *E. coli* that are mostly responsible for diarrhea and other clinical complications ([Islam et al., 2013](#); [Huang et al., 2006](#)). Therefore, 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](#)).

Ruminants are natural reservoirs of many pathogenic strains of *E. coli* and play momentous role in the epidemiology of human infections. The bacterium is harbored in the GIT and excreted out through feces. The pathogen could be attached in the skin or any other parts of the body through fecal contamination, and may contaminate the carcass during evisceration or other sort of manipulation. This will further enhance spillover of pathogenic *E. coli* to human ([Islam et al., 2013](#)).

Acquisition and development of antimicrobial resistance among enteric *E. coli* is a global public health fear, and is becoming worse day by day in communities and healthcare settings. Frequent use of antimicrobial agents both in human and animal medicine along with various risk factors enhancing the global burden of drug resistant bugs ([Hasan et al., 2014](#); [Shaikh et al., 2015](#)). The

horrifying situation might be corrected through minor treatment regimen or surgical manipulations ([Van Boeckel et al., 2015](#)). Moreover, antimicrobial resistance affects animal health and environment negatively that is in line with the One Health concept which illustrates interconnection among animal, human and environmental health. Livestock in particular, are considered as the key spreader of antimicrobial resistant *E. coli*, and are frequently associated with public health spillover ([McEvoy et al., 2003](#); [Dulo et al., 2015](#)). Therefore, the study was aimed to demonstrate the epidemiology and antimicrobial resistance patterns of *E. coli* in goats in a costal tourist spot, Cox's Bazar, where many peoples travel from different corners of the globe each year.

MATERIALS AND METHODS

Study population, sample collection and transportation: Rectal swab samples (n=150) were collected from goats admitted to the Upazilla Veterinary Hospital (UVH), Cox's Bazar during August 2013 to June 2014. The goats were selected randomly based on willingness of the owner to test their animals for *E. coli* infection. The study protocol was permitted by ethics committee of the Chittagong Veterinary and Animal Sciences University. The enrolled animals have had a history with one or more of the clinical sign(s) such as high fever, diarrhea, pneumonia, peri and post-partum complications etc.

Animal owners have been interviewed and information on age, sex, body condition score (BCS), breed, other demographic and management related practices were recorded using a structured questionnaire. BCS was defined through four qualitative descriptors which characterize worst to best physical conditions of the selected goat population. Samples were collected by inserting sterile swabs into rectum of goats. The collected swabs were placed in a falcon tube (5 mL) containing Stuart's transport media (Oxoid, Basingstoke, Hampshire, UK), and using an ice box the samples were sent to the Microbiology Laboratory and the Poultry Research and Training Centre (PRTC) Laboratory, CVASU for microbiological examinations.

Plating, identification and characterization of *E. coli*: Pre-enrichment was conducted using buffered peptone water (BPW) broth (Oxoid Ltd., Basingstoke, Hampshire, UK), as described by [Thaker et al. \(2013\)](#). A loop-full of enriched bacterial culture was inoculated onto MacConkey (Oxoid Ltd., Basingstoke, Hampshire, UK) agar. Large pink colonies attained from the MacConkey agar plates were subsequently inoculated

onto Eosin Methylene Blue (EMB) (Oxoid Ltd., Basingstoke, Hampshire, UK) agar to verify whether the isolates were *E. coli* or not. The EMB dyes react with products released from lactose or sucrose by *E. coli* used as carbon and energy sources, developing green metallic sheen considered as positive for *E. coli* ([Virpari et al., 2013](#); [Nazir et al., 2005](#); [Tanzin et al., 2016](#)).

Grams staining technique was used to determine morphology and staining characters. The suspected colony on EMB agar was stained as described in OIE Manual of Standards for Diagnostic Tests and Vaccines ([Hunter, 1998](#)). All culture positive isolates (n=89) were inoculated onto nutrient agar (NA) and incubated for 24 h at 37°C. The bacteria were subjected to various biochemical analyses including carbohydrate fermentation, catalase and indole tests for final confirmation.

Microbial sensitivity analysis of isolated *E. coli*: The *E. coli* isolates were examined for the diversity in antibiogram analysis by disk diffusion method on Mueller-Hinton agar, as described by [Bauer et al. \(1966\)](#). A bacterial turbidity corresponding of 0.5 McFarland standards was used for each isolate. The 0.5 McFarland standard was prepared by adding 0.5 mL of 1% BaCl₂.2H₂O (11.75g/L) to 1%, 99.5 mL of (0.36N) H₂SO₄ ([Carter., 2012](#)).

The groups and subclasses of antimicrobials used for *E. coli* along with zone of inhibition (diameter) to be considered as resistant (R), intermediately resistant (I) and sensitive (S) as recommended by Clinical and Laboratory Standards Institute ([CLSI, 2007](#)).

Data analysis: Data including age, sex, breed, body condition score and transportation history etc. were taken from the goats clinically examined and subsequently entered into MS excel (Microsoft office excel-2013, USA). Data management and data analysis were done by STATA version-14 (STATA Corporation, College Station, Texas). The prevalence of *E. coli* associated with different categorical variables compared for statistical significance using chi-square (χ^2) test. A *P* value of ≤ 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

E. coli is an important bacterial agent that roots great economic losses in the farming industry. One of the major challenging public health issues is unnecessary and recurrent use of antimicrobial agents associated with development, carriage and the theatrical spread of antibiotic resistant bacteria, which is fascinating. *E. coli* carrying antimicrobial resistant genes can cross species barrier and confer resistance to commonly used antimicrobial agents like penicillins, tetracycline,

gentamicin, cephalosporins, and carbapenems (last antibiotic resort), as documented by [Hasan et al. \(2014\)](#). Recently, resistance mechanism has been discovered against colistin which is considered as last antibiotic resort when all other available treatment options would become inadequate ([Liu et al., 2015](#)).

In this study, the isolated *E. coli* were subjected for antibiogram analysis to determine the microbial sensitivity patterns. Based on colony characteristics of *E. coli* on different media, the bacteria were identified. On NA, the *E. coli* produces smooth, circular, white to greyish white colonies. On EMB agar, the colonies were smooth, large, circular, blue-black colonies with green metallic sheen. On MacConkey agar, the bacteria produce smooth pink colonies. The *E. coli* isolates fermented five sugars (dextrose, lactose, sucrose, maltose and mannitol) producing both acid and gas. Hydrogen peroxide was broken down into water and oxygen in catalase test. The bacteria showed positive reaction in indole test showing red or red-violet color on the top layer of pure bacterial culture broth. These findings were similar to other published reports ([Beutin et al., 1993](#); [McKee et al., 1995](#); [Abdullah et al., 2010](#)).

Of 150 swab samples, 52.0% (n=78/150; 95% CI; 43.70-60.21) were found to be positive in the panel bacteriological and biochemical tests, and were confirmed as *E. coli* (**Table 1**). The result was supported by the findings of [Abdullah et al. \(2010\)](#) and [Türkyılmaz et al. \(2014\)](#) who found that 60.0% and 36.4% samples were positive for *E. coli*. However, the little variation in the prevalence might be due to sampling variation, climatic and geographical diversity of the goats examined.

To demonstrate the association of various risk factors, 9 risk practices were studied where 5 were significantly associated with *E. coli* prevalence in goats. The prevalence of *E. coli* infection was 65.0% (95% CI, 53.52-75.33) that was found to be significantly higher (*P*=0.00) in young kids (3 to 12 months) as compared to adult goats. The prevalence of *E. coli* infection in kids were closely linked with the findings of [Zaki et al. \(2010\)](#) who found 68.84% prevalence in goat kids. The reasons behind higher prevalence in kids might be due to lack of sufficient acquired immunity, susceptibility to different *E. coli* pathotypes, nutritional imbalance, faulty husbandry practices and unhygienic condition of the farms ([Zaki et al., 2010](#); [Jafari et al., 2012](#)).

A significant association (*P*=0.00) has been found with hygienic condition of the farm where goats were reared, and poor hygienic status showed higher prevalence (66.7%; 95% CI; 53.66-78.04) than good and moderate

status. Farm hygiene is compromised ruthlessly in many developing and middle-developed countries like Bangladesh where different species, both sick and healthy animals are kept in the same premises with high animal density. Therefore, poor hygiene is one of the supporting factors in *E. coli* prevalence (Islam et al., 2008; Rehman et al., 2014).

E. coli prevalence was significantly ($P=0.01$) higher (64.8%; 95% CI; 50.62-77.31) in goats having the history of recent transportation than those did not. Transportation through vehicles or other means and force animals to walk may enforce stress reducing immunity to infectious agents leading to fecal carriage and shedding of *E. coli* (Minihan et al., 2003; Islam et al., 2014). There were significant association between *E. coli* prevalence and goats having diarrhea. The prevalence was higher (62.0%; 95% CI; 50.40-72.71) in diarrheic goats as compared to those do not have diarrhea. Bacterial diarrhea upsetting kids-goat occurs mostly in intensive breeding systems where pens, paddocks, and interior kids-goat sheds are occupied. *E. coli* appears to be the prevailing enteropathogens which pose significant role in the occurrence of diarrhea in goat kids (Zaki et al., 2010).

Pond and municipality supplied water were highly significant ($P=0.00$) with *E. coli* prevalence. The main reason might be due to environmental contamination of water sources (Patoli et al., 2010; Rashid et al., 2015).

Bacteriologically and biochemically confirmed isolates were analyzed to determine the antimicrobial susceptibility patterns. High level of resistant isolates were found against Ampicillin (AMP) (65.38%; n=51/78) and Amoxicillin-clavulanic Acid (AML) (60.26%; n=47/78), followed by Trimethoprim-sulfamethoxazole (SXT) (52.56%; n=41/78), Tetracycline (TE) (51.28%; n=40/78), Streptomycin (S) (47.44%; n=37/78) and Gentamicin (CN) (37.18%; n=29/78) as described in **Table 2** and **Figure 1**. Least degree of resistance was noted against Ceftriaxone (CRO) (21.79%; n=17/78) and Cefotaxime (CTX) (26.92%; n=21/78).

E. coli isolates were highly sensitive to Ceftriaxone (CRO) (50%; n=39/78), Cefotaxime (CTX) (39.74%; n=31/78) and Gentamicin (CN) (46.15%; n=36/78). Present antibiogram profile of *E. coli* where maximum number of isolates were resistant to AMP, AML, SXT, TE, S and CN; these findings were in consistent with other reports

Table 1: Association of different categorical variables with the prevalence of *E. coli* in goats

Sl. No.	Variable	Category (N)#	Positive (n)	% Prevalence (95% CI)	P-value
1	Breed	BBG (43)	23	53.5 (37.65-68.82)	0.56
		CB (73)	35	47.9 (36.10-59.96)	
		JP (34)	20	58.8 (40.69-75.35)	
2	Age	Young (3-12m) (80)	52	65.0 (53.52-75.33)	0.00*
		Adult (>12m) (70)	26	37.1 (25.88-49.52)	
3	Hygienic status	Good (28)	11	39.3 (21.50-59.42)	0.00*
		Moderate (59)	25	42.4 (29.60-55.93)	
		Poor (63)	42	66.7 (53.66-78.04)	
4	Sex	Male (79)	38	48.1 (36.71-59.63)	0.31
		Female (71)	40	56.3 (44.04-68.08)	
5	History of recent transportation	Yes (54)	35	64.8 (50.62-77.31)	0.01*
		No (96)	43	44.8 (34.62-55.28)	
6	Season	Wet (86)	48	55.8 (44.69-66.52)	0.28
		Dry (64)	30	46.9 (34.27-59.76)	
7	Diarrhea	Yes (79)	49	62.0 (50.40-72.71)	0.01*
		No (71)	29	40.8 (29.31-53.15)	
8	Body Condition Score (BCS)	CPB (21)	10	47.6 (25.71-70.21)	0.86
		CSP (64)	34	53.1 (40.23-65.72)	
		GBC (24)	14	58.3 (36.64-77.89)	
		RBC (41)	20	48.8 (32.87-64.86)	
9	Source of drinking water	Pond (11)	7	63.6 (30.79-89.07)	0.00*
		Tube-well (56)	20	35.7 (23.35-49.64)	
		Municipality (83)	51	61.4 (50.11-71.93)	
Total		150	78	52.0 (43.70-60.21)	

(N)#, total observation in each category; *Significance was determined when $P \leq 0.05$; CI, Confidence Interval; BBG, Black Bengal goat; CB, Crossbred; JP, Jamunapari; GBC, Good Body Condition; CPB, Cachectic, protruding rib with prominent pin bone; CSP, Cachectic (severe), protruding rib with prominent pin bone; RBC, Rough Body Condition

Table 2: Antimicrobial resistance pattern of *E. coli* isolates in goats

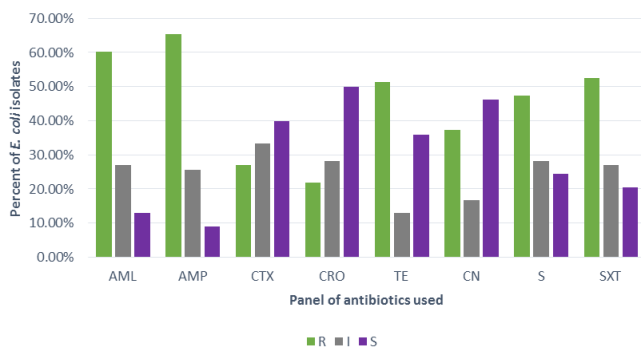
Antibiotics	No. of isolates tested	Resistant (%)#	Intermediately resistant (%)#	Sensitivity (%)#
Amoxicillin-clavulanic Acid (AML)	78	47 (60.26)	21 (26.92)	10 (12.82)
Ampicillin (AMP)	78	51 (65.38)	20 (25.64)	7 (8.97)
Cefotaxime (CTX)	78	21 (26.92)	26 (33.33)	31 (39.74)
Ceftriaxone (CRO)	78	17 (21.79)	22 (28.21)	39 (50)
Tetracycline (TE)	78	40 (51.28)	10 (12.82)	28 (35.9)
Gentamicin (CN)	78	29 (37.18)	13 (16.67)	36 (46.15)
Streptomycin (S)	78	37 (47.44)	22 (28.21)	19 (24.36)
Trimethoprim-sulfamethoxazole (SXT)	78	41 (52.56)	21 (26.92)	16 (20.51)

#Number in parenthesis indicates percentage

Table 3: Multi-drug resistance (MDR) profile of *E. coli* based on phenotypic microbial sensitivity analysis

Phenotypes of antibiotic resistance	Number of antimicrobial subclasses resistant to	Number of isolates (n)
AMP-AML-S	3	1
AML-TET-S	3	1
AMP-AML-TET-S	4	1
AMP-AML-TET-CN	4	1
AMP-AML-TET-SXT	4	1
AMP-TET-SXT-CN	4	1
AMP-AML-S-CN	4	1
AML-TET-S-SXT	4	1
AMP-AML-S-SXT	4	2
AMP-TET-S-SXT	4	1
TET-S-SXT-CN	4	1
AMP-AML-TET-S-SXT	5	1
AMP-TET-S-SXT-CN	5	1
AMP-AML-CTX-TET-CN	5	2
AMP-AML-S-SXT-CN	5	1
AMP-AML-CTX-CRO-S	5	1
AMP-AML-TET-S-CN	5	1
AMP-AML-CRO-S-SXT	5	1
AMP-CRO-TET-S-SXT-CN	6	1
AMP-AML-TET-S-SXT-CN	6	1
AMP-AML-CTX-TET-S-SXT	6	1
AMP-AML-CRO-S-SXT-CN	6	1
AMP-AML-CTX-TET-S-CN	6	1
AMP-AML-CTX-CRO-S-SXT-CN	7	1
AMP-AML-CTX-CRO-TET-S-SXT	7	2
AMP-AML-CTX-CRO-TET-S-CN	7	1
AMP-AML-CTX-CRO-TET-S-SXT-CN	8	2
Total		31

AML (*Amoxicillin*); AMP (*Ampicillin*); CTX (*Cefotaxime*); CRO (*Ceftriaxone*); TE (*Tetracycline*); CN (*Gentamicin*); S (*Streptomycin*); SXT (*Trimethoprim-Sulfamethoxazole*)

**Figure 1:** Percent of isolates showing resistant, intermediately resistant and sensitive to the antimicrobials tested. AML (*Amoxicillin*); AMP (*Ampicillin*); CTX (*Cefotaxime*); CRO (*Ceftriaxone*); TE (*Tetracycline*); CN (*Gentamicin*); S (*Streptomycin*); SXT (*Trimethoprim-Sulfamethoxazole*)

(Islam et al., 2013; Bist et al., 2014; Ansari et al., 2014). Gram negative bacteria are commonly resistant to Penicillin now and resistance pattern becoming frequent to β -lactam group of antibiotics which is dismaying. Furthermore, development of resistance against clinically effective groups of antimicrobials like Tetracycline, Aminoglycosides and Folate pathway inhibitor is a horrifying news both for human and animal health.

Of 78 *E. coli* isolates, 31 isolates were found to be resistant to minimum 3 to maximum 8 sub-classes of antibiotics; these were considered as multidrug resistant (MDR) *E. coli* shown in **Table 3**, as reported by Hasan et al. (2014). High degree of MDR *E. coli* emergence depicts a critical situation where it would be very difficult to manage clinical conditions arises due to infectious *E. coli*.

Perhaps, food animals may transmit AMR *E. coli* bugs (strains) to human through close human-animal interaction and subsequently posing grave zoonotic consequences.

Fascinatingly, a minor percentage of *E. coli* were found as resistant to Cepheims group of antibiotics (e.g., Cefotaxime and Ceftriaxone) which are rarely used in veterinary practices in Bangladesh. Though the percentage is small, the resistance patterns are growing against those rear group of drugs. This might be due to close human-animal interface through environmental cross contamination ([Islam et al., 2013](#); [Rashid et al., 2015](#)).

CONCLUSION

Current research finding reveals a high level of MDR *E. coli* emergence in hospital settings which is a serious public health issue of concern. Many escalating factors are contributing to the spread of antibiotic-resistant superbugs in the environment. To prevent and control drug resistant pathogens, it is critical to design and implement fast detection techniques with maximum accuracy in addition to safeguarding the best use of existing drugs.

CONFLICT OF INTEREST

Nothing to declare.

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