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CULTURAL AND BIOCHEMICAL CHARACTERIZATION OF SHEEP *Escherichia coli* ISOLATED FROM IN AND AROUND BAU CAMPUS

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ABSTRACT

Pathogenic *Escherichia coli* remain as an important etiological agent of sheep diarrhoea in Bangladesh. The present study was designed for the cultural and biochemical characterization of Sheep *E. coli* from diarrhoeic and apparently healthy sheep in and around BAU campus for the period from January to October, 2007. Out of 90 faecal samples, 36 from diarrhoeic and 54 from apparently healthy sheep collected from different areas in and around BAU campus, 15 (41.67%) and 21 (38.38%) were found to be positive for *E. coli*. The cultural characterization of all positive sheep *E. coli* revealed greenish black colony with metallic sheen in Eosine methylene blue agar, bright pink color smooth transparent colony in MacConkey agar, green color colony in Brilliant green agar, slight pinkish smooth colony in Salmonella-Shigella agar and colorless colony with hemolysis in blood agar. In case of biochemical characterization, all of the isolates showed fermentation of dextrose, sucrose, fructose, maltose and mannitol with the production of acid and gas, negative result to Voges-Proskaure test, positive result to Methyl-red test and differential result to Indole test.The overall prevalence of *E. coli* was recorded as 80.05% through the cultural and biochemical characterization. The antibiotic sensitivity and resistance pattern showed that the isolates of sheep *E. coli* were highly sensitive to ciprofloxacine, co-trimoxazol, nalidixic acid and chloramphenicol but to the erythromycin the isolates were highly resistant.

Key words: *E. coli*, sheep, cultural, biochemical, characterization

INTRODUCTION

E. coli is a multitalented, enteric Gram-negative bacillus, and best known as a noninvasive commensal that grows in mass culture in human and in animal gut lumen, perhaps keeping other more harmful bacteria away from proliferating (Buxton and Fraser, 1977). Lambs are vulnerable to *E. coli* infection. Two age groups appear to be susceptible, lambs of 1-2 days of age and lambs of 3-8 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 sheep is ranged from 1-5% with an age distribution of 3-12 weeks old (Mason and Carbould, 1981). Due to *E. coli* infection in sheep wool production, meat production is declined dramatically. As a result the farmers who are economically dependent on sheep rearing become looser. Cultural characterization of *E. coli* by using different media and biochemical characterization by observing variable reaction to different sugars & chemicals are the basic rules for their identification. Antibiotics are widely used in case of diseased animal in the treatment of Sheep 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. Various parameters including the prevalence, isolation, identification and epidemiological investigation of *E. coli* was studied by Choudhury *et al*., 1967; Rahman *et al*., 1987; Amin *et al*., 1988; Nazir, 2004; Hasina, 2006 and Zinnah, 2007. Amin *et al*., 1987 studied on *E. coli* in calves. Hasina, 2006 studied on enteropathotypes of *E. coli* from diarrhoeic calves. Zinnah, 2007 conducted an experiment on *E. coli* isolated from human, cattle, sheep, goat, chickens, duck, pigeon, drain sewages and soil.For the prevention and control of any microbial disease, prior isolation, identification and characterization of that particular etiological agent in a country is a pre-requisite. Considering the above facts the present study was undertaken with some distinct aims these are isolation, identification, biochemical characterization and antibiotic sensitivity pattern of *E. coli* isolated from sheep faeces.

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MATERIALS AND METHODS

The study was conducted in the Bacteriology laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh during the period of January 2007 to October 2007.

Selection of experimental area, experimental animal and collection of samples

Areas selected for the experimental study were confined in and around BAU, Mymensingh campus. The selected areas were BAU Sheep and Goat Farm, BAU Veterinary Clinic, BAU Nutrition field and Boyra, BAU Campus. Sheep having diarrhea and apparently healthy sheep were selected for the experimental study. A total number of 90 field samples comprising rectal swabs were aseptically collected into nutrient broth (NB) from different spots of BAU campus and around the campus and carried to the laboratory for the characterization of *E. coli.* Out of 90 samples 36 were collected from diarrhoeic sheep and 54 were collected from apparently healthy sheep.

Cultivation and Isolation

Primary growth of all kinds of bacteria was performed in NB. Rectal swabs were collected with sterile cotton bud by gentle touch, and then inoculated into the NB, incubated for overnight at 37°C to obtain the primary culture. After primary culture of the organism smears were prepared from each of the test tubes and the smears were fixed. The fixed smears were stained with Gram's Method of staining and examined under microscope at 100 magnifications using immersion oil. In presence of gram negative rods in the smears, the materials from the tube corresponding to the smears were streaked into MacConkey agar, Eosin-Methyline Blue agar, Salmonella-Shigella agar and Brilliant green agar separately. The plates were then incubated for overnight at 37°C and the plates containing characteristic colonies of *E. coli* were selected for subculture. Motility test and Gram's staining testare performed to identify the plates containing *E. coli* accurately. Subculturing in Eosin - Methyline Blue agar was performed from the suspected plates containing *E. coli* obtain a pure culture. These pure isolates obtained in this way were used for further study. (Cheesbrough, 1984).

Morphological Characterization by Gram's staining method

A small colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gently heating. Crystal violet solution was then applied on the smear to stain for two minutes and then washed with running water. Lugol's iodine was then added to act as mordant for one minute and then again washed with running water. Acetone alcohol was then added, which act as a decolorizer, for 5 seconds. After washing with water, safranine was added as counter stain and allowed to stain for two minutes. The slide was then washed with water, blotted and dried in air and then examined under microscope with high power objectives (100X) using immersion oil (Merchant and Packer, 1967).

Motility test using hanging drop slide

The motility test was performed according to the method described by Cowan (1985) to differentiate motile bacteria from the non-motile one. Before performing the test, a pure culture of the *E. coli* isolates was allowed to grow in NB. One drop of cultured broth was placed on the cover slip and placed inverted 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 cover-slip to prevent air current and evaporation of the fluid. The hanging drop slide was then examined carefully under 100x objective of a compound microscope using immersion oil. The motile and non-motile organisms were identified by observing motility in contrasting with to and fro movement of bacteria. The motile bacteria with to and fro movement were identified as *E. coli.*

Sugar fermentation test

The carbohydrate fermentation test was performed by inoculating a loop full of nutrient broth culture of the organisms into the tubes containing different sugar media (five basic sugars such as dextrose, sucrose, lactose, maltose and mannitol) and incubated for 24 h at 37°C. Acid production was indicated by the color change from reddish to yellow in the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes (Cheesbrough, 1984).

Indole test

Typical colonies were inoculated into individual tube of 2 ml tryptone water, incubated at 37°C for up to 24 h and tested for indole production with Kovac's reagent. If the reagent showed cherry red color layer then it confirms the positive test, which indicate the presence of faecal coliform (*E. coli*) group (Cheesbrough, 1984).

Methyl-Red test

The test was performed by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth (as used in the VP test). After overnight incubation at 37°C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of bright red color, indication of acidity (Cheesbrough, 1984).

Voges Proskauer test

An amount of 2 ml of sterile glucose phosphate peptone water were inoculated with the 5ml of test organisms. It was incubated at 37°C for 48 h. A very small amount (knifepoint) of creatine was added and mixed. Three milliliters of sodium hydroxide were added and shake well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In negative cases there was no development of pink color (Cheesbrough, 1984).

Antibiogram study

Susceptibility of *E. coli* isolates to different antibiotic agents was determined *in vitro* by employing a modified disc diffusion test of the Kirby-Bauer (Bauer *et al.,* 1966) method. It is frequently employed to demonstrate the drug sensitivity of microorganisms isolated from infectious process and to interpret their disease potential. The procedure involved measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Commercially available antimicrobial discs (Mast Diagnostics, Merseyside, U.K.) were used fro the test. A suspension of test organism was prepared in nutrient broth by overnight culture. With a sterile pipette 1 ml of test culture was poured on EMB agar plate. Sterile glass spreader was used to spread the culture homogenously on the medium. Antibiotic discs were placed aseptically the surface of the inoculated plates applying appropriate special arrangement with the help of a sterile forceps. The plates were inverted and incubated at 37°C for 24 h aerobically (Carter, 1979).

RESULTS AND DISCUSSION

In the present study, out of 90 rectal swab samples, 37 samples were identified as *E. coli* in which 15 were collected from diarrhoeic sheep and 21 were collected from apparently healthy sheep belonging to different areas in and around BAU campus. This study revealed that the higher prevalence rate was from diarrhoeic sheep (41.67%) and the lower from apparently healthy sheep (38.88%) (Table 1).

Table 1. Demonstration of overall prevalence percentage of *E. coli* in diarrhoeic and apparently healthy sheep

SL. No.	Sources of samples	Number of samples examined	Culture Examination		Biochemical Examination		Number of isolates of *E. coli*	Percentage of prevalence
			Positive for *E. coli*	Negative for *E. coli*	Positive for *E. coli*	Negative for *E. coli*		
1.	Diarrhoeic sheep	36	15	21	15	21	15	41.67
2.	Apparently healthy sheep	54	21	33	21	33	21	38.88

The cultural characterization of all positive sheep *E. coli* revealed greenish black colony with metallic sheen in Eosine methylene blue agar, bright pink color smooth transparent colony in MacConkey agar, green color colony in Brilliant green agar, slight pinkish smooth colony in Salmonella-Shigella agar and colorless colony with hemolysis in blood agar (Table 2) which were similar to the findings of other authors (Buxton and Fraser, 1977; Hasina, 2006 and Nazir, 2004). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired and after motility test we also found that they were motile (Table 2).

Table 2. Results of cultural, morphological and motility characteristics of the isolated *E. coli*

Sources of isolates	Colony characteristics					Staining character	Motility
	EMB agar	MC agar	BG agar	SS agar	Blood agar		
Faecal samples of BAUSGF	Greenish black colony with metallic sheen	Bright pink color smooth transparent raised colonies	Green color colony	Slight pinkish smooth colonies	Colorless colony with hemolysis	Pink short rod, Gram negative bacilli	+
Faecal samples of BAUVC	Do	Do	Do	Do	Do	Do	+
Faecal samples of BBAUC	Do	Do	Do	Do	Do	Do	+
Faecal samples of BAUNF	Do	Do	Do	Do	Do	Do	+

Legends
EMB=Eosine-methylene blue **MC** = MacConkey **BG**= Brilliant Green
SS = Salmonella-Shigella + **=** Positive
BAUSGF = Bangladesh Agricultural University Sheep &Goat Farm**, BAUVC =** Bangladesh Agricultural University Veterinary clinic, **BBAUC =** Boyra, Bangladesh Agricultural University Campus, **BAUNF =** Bangladesh Agricultural University Nutrition Field

In biochemical examination all the isolates fermented dextrose, sucrose, fructose, maltose and mannitol with the production of acid and gas within 24-48 hrs of incubation. The isolates also revelaed positive reaction in MR test, negative reaction in VP test and differential results in Indole test.
In the present study we found that the isolates of sheep *E. coli* was highly sensitive to ciprofloxacin, co-trimoxazol, nalidixic acid and chloramphenicol while moderately sensitive to kanamycine and amoxicillin and less sensitive to cephalexin. The isolates of sheep *E. coli* were fully resistant (100%) to erythromycin (Fig. 1).

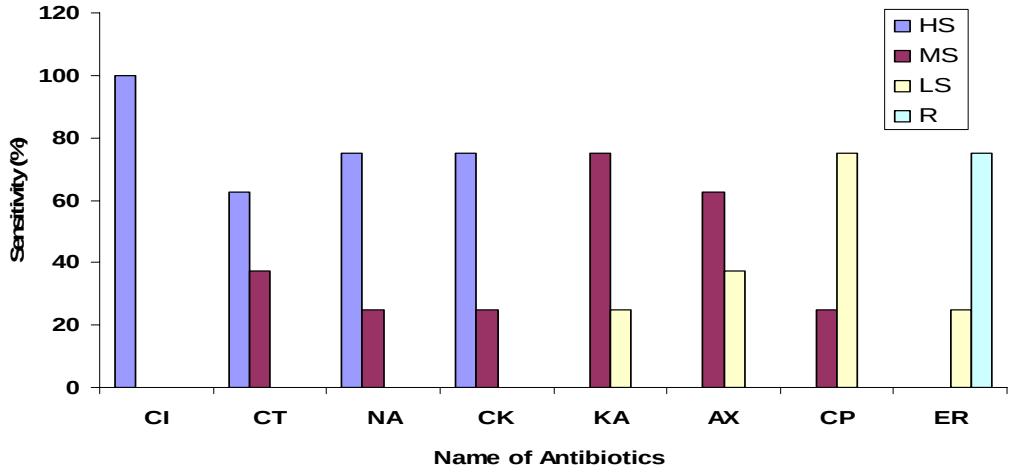

Figure 1: Overall antibacterial sensitivity and resistance pattern of the isolated sheep *E. coli*

Legends
HS = Highly sensitive, **MS** = Moderately sensitive, **LS** = Less sensitive,
CP =Cephalexin **KA** = Kanamycin **CT** = Co-trimoxazol
NA = Nalidixic acid **CK** = Chloramphenicol **CI** = Ciprofloxacin
AX = Amoxicillin **ER** = Erythromycin **R** = Resistant

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