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Escherichia Coli from Day Old Chicks of a Selected Breeder Farm in Bangladesh

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E. coli infection is a very common problem encountered among newly hatched chicken in Bangladesh. The aim of present study was to detect and characterize E. coli in the gut, liver and lungs of Day Old Chicks (DOC). Total viable count (TVC) in gut, liver and lung was the highest among "C" grade chicks compared to those of "A" and "B" grade chicks. The isolated E. coli were sensitive to Enrofloxacin and Ciprofloxacin but resistant to Cloxacillin, Nalidaxic acid and Erythromycin. The result indicated that load of E. coli was higher among the lower grade chicks.

KEY WORDS
E. coli, total viable count, Bangladesh, antibiotic resistance, grade of chickens.

INTRODUCTION

There are 3867000 poultry breeders rearing about 311458000 chicks per year that are obtained from 227 hatcheries operating in Bangladesh (BBS Survey, 2010). All basic production operations in the poultry industry begin in a hatchery. Key stages from primary breeding through parent breeder production to multiplication of egg or meat strain day-old commercial chicks or Parent Stock (PS) chicks, largely depend on breeder farms quality and the hatching process (Windhorst et al, 2008). Bacterial load in Day Old Chicks (DOC) mainly depend on breeder performance, such as location and sanitary & hygienic condition of the parent's stock farm, ventilation, bio-security system, prevention and control measures for microbial diseases with antibiotic therapy and proper vaccination program, egg fumigation, egg storage and hatchery conditions.

The bacterial count in poultry housing systems is particularly high in comparison to those of pig and cattle. Infections gain entrance to a flock from various sources. Morbidity of 32.38% with a mortality rate of 21.30% was recorded mainly due to bacterial diseases among chickens. (M. Z. Uddin et al, 2011). The bacterial load of DOC in the vital organs like liver, lungs, heart, trachea and gut mainly comes from breeder flock which is not properly vaccinated against major bacterial diseases like Salmonellosis, Fowl cholera, Infectious Coryza, Fowl Typhoid. One of the major constrains in the development of poultry industry is that the chicks are immunologically weak and prone to rapid and persistent colonization by many pathogenic and beneficial bacteria during the first 2 weeks of life (Barrow et al, 1988). It is well established that the gastrointestinal normal microflora play an important role in the health and wellbeing of chicks. Various pathogenic microbes, such as *E. coli,* Streptococcus ssp., Pasteurella ssp., Staphylococcus ssp., Bacillus ssp. and Salmonella ssp., have been implicated to reduce the growth of poultry. Possible mechanisms for this reduction of growth are toxin production, utilization of nutrient essential to the host, production of diseases and suppressions of microbes that synthesize vitamins or other host growth factors (Duke et al, 1986).

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Adequate literature is available regarding the studies on colibacillosis in poultry birds but information about the study on *E. coli* of DOC is very meager (Islam et al, 2009, Islam et al, 2012). Present study was designed, to isolate and identify the bacterial flora in vital organs like liver, lungs and gut of healthy (A grade), weak (B grade), culled (C grade) chicks obtained immediately after hatching, to determine the Total Viable Count (TVC) of bacteria in different organs of the three grade of chicks with antibiotic sensitivity pattern.

MATERIALS AND METHODS

DOC of different groups were collected from a Breeder farm of Valuka, Mymensingh where vaccination schedule against bacterial diseases was strictly followed and sanitary conditions were well maintained. DOCs were carried to the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh. A total of eighteen DOCs were collected from three different batches (Batch 1, 2 and 3) of breeder flock of different ages. Each batch having 6 chicks with three grades (Grade A, B and C) having 2 birds of each grade. Gut, liver and lungs were used as samples for isolation and identification of bacteria. Lung sample were collected from bird by using sterilized cotton swabs. The gut and livers were incised and the samples were then collected using a sterile inoculating loop.

After collection, the samples were inoculated into nutrient and selenite broth and then incubated at 37^{o}C for 24 hrs. After 24 hours the incubated broth was streaked onto BAU made Nutrient agar, Blood agar, MacConkey (MC) agar, Salmonell-Shigella agar and Eosin Methylene Blue (EMB) agar. Gram's staining was performed to study their morphology and motility test (Cowan et al, 1985) was conducted to differentiate motile bacteria from non-motile bacteria. For biochemical test, stock nutrient broth was taken for sugar fermentation test in five basic sugars like dextrose, lactose, sucrose, maltose and mannitol to observe the production of acid and gas. Other biochemical tests used were: indole test, MR-test and VP-test. Total Viable Count (TVC) was done on nutrient agar following ten-fold dilution, where 0.1 ml of each dilution was transferred to NA media. Following incubation, plates exhibiting 20-300 colonies were counted forming units per gram (CFU/gm) of sample. Lastly antibiotic sensitivity test was conducted to know the drug sensitivity and resistance pattern. Antimicrobial discs commercially available (Mast Group Ltd, Merseyside, UK) in the market were used for the test to determine the drug sensitivity pattern of *E. coli*. Susceptibility and resistance of different antibiotics was measured in vitro by employing the modified Kirby-Bauer method (Bauer et al, 1966).

RESULTS

E. coli was isolated from gut, liver and lungs of DOC (Table 1). In case of batch 1 *E. coli* was not detected in the liver and lung of grade A and B. However the organism was detected in the gut of grade A and in both gut and liver of Grade B chickens of batch 2, and in gut of grade A, and both gut and liver of grade B in batch 3. *E. coli* was also found in gut, liver and lung of grade C DOC of all batches.

Total viable count of gut in "A" grade chicks was 2.3×10^{6}, in "B" grade 2.6×10^{6}, in "C" grade 2.9×10^{6} and the mean was 2.6×10^{6}. The highest TVC was found in the gut of "C" grade chicks. The TVC of liver in "A" grade chicks was 2.6×10^{5}, in "B" grade 2.7×10^{5}, in "C" grade 2.8×10^{5} and the mean was 2.6×10^{5}. The lungs showed a TVC of 2.2×10^{4} in "A" grade chicks, 2.3×10^{4} in "B" grade and 2.5×10^{4} in "C" grade. The mean was 2.3×10^{4} (Table 2).

On MC agar, bacterial colonies were found as dark pink colored raised colonies (Fig 1). EMB agar plates were streaked separately with the organism after growing in NB and revealed the growth of bacteria after 24 hours of incubation at 37^{0}C and growth of bacteria was indicated by the presence of smooth, moist circular colonies with bright metallic sheen (Fig 1). Growth on SS agar media was indicated by pink color smooth colony (Fig 1). Non haemolytic circular colony was observed in Blood agar (Fig 1).

E. coli showed Black color colony with metallic sheen in EMB media, Bright pink colored, smooth colonies in MC agar and pink color smooth colony in SS agar as a Cultural characteristics. It also showed staining

properties of Gram negative, pink color, small rod shaped appearance arranged in single or chain and the isolate was motile as morphological characteristics. In Blood agar it showed non hemolytic circular colony only as a cultural characteristics but no morphological characteristics.

The microscopic examination of Gram's stained smears from agar media revealed Gram-negative pink colored rod shaped, arranged in single or in pairs. All of the isolates were motile with hanging drop slide.

The isolate fermented dextrose, maltose, mannitol, lactose and sucrose with acid and gas production. Acid production was marked by the color change from reddish to yellow and the gas production was indicated by the presence of gas bubbles in the inverted Durham's to be kept inside each of the test tubes containing sugar media were presented in the Table 3 and Fig 3. The isolate was indole positive (Fig 3). The isolate was MR positive and VP negative

Isolated *E. coli* were tested for antibiotic sensitivity against commonly used antibiotics. Zone of inhibition varying from 10-29 millimeter were characterized as resistant (-), moderately sensitive (++) and highly sensitive (+++). The results are presented in the Table 4-6. From the antibiogram study it was revealed that out of 6 *E. coli*, 11.3% *E. coli* were resistant, 61.1% were less sensitive and 27.6% were moderately sensitive to Amoxicillin. 12.1% of the isolates were resistant, 43.7% were less sensitive, 22.1% moderately sensitive and 22.1% highly sensitive to Enrofloxacin. 33.7% *E. coli* was highly sensitive, 50% moderately sensitive, 16.3% less sensitive to Ciprofloxacin. 33.3% isolates were moderately sensitive and 66.7% were less sensitive to Cephalexin.33.3% *E. coli* resistant to Erythromycin. None of the isolates were resistant to Kanamycin. However, 1 isolate was resistance to Nalidixic acid (Table 5). The isolated *Escherichia Coli* were highly sensitive to Enrofloxacin, Ciprofloxacin, moderately sensitive to Cephalexin, Amoxacillin, and resistant to Cloxacillin, Nalidixic acid and Erythromycin (Table 5-6, Fig 2).

DISCUSSION

The bacterial load was found higher in gut followed by liver and lungs. These findings partially agree with the findings of (Maiorka et al, 2006).

Results of biochemical tests used for the isolation and identification of the bacteria coincided with the results of (Buxton and Fraser 1977) and Cowan (1985) who described that the isolated *E. coli* fermented five basic sugars with production of acid and gas. The organism formed indole and gave a positive methyl red test and negative in Voges-Proskauer reaction which is presented in the Table 3 and in Fig 3.

Morphology, staining and cultural characteristics of the bacteria in different cultural media as recorded in the study were almost similar as reported by K. A. Choudhury et al, (1985) who reported that staining and morphology of the isolated *E. coli* exhibited Gram negative, small rod, arranged in single or pairs, non-spore former.

Although some isolates of *E. coli* from DOC were found pathogenic. However, they did not produce disease in the chicks. The disease is the outcome of interaction of host, agent and environment. It is reported that more than one predisposing factors such as environment and management factors (housing, climate), imbalance nutrition, immune status of the poultry might help in the production of diseases along with the presence of bacteria.

Antibiogram study of the present study revealed that *E. coli* was highly sensitive to Enrofloxacin, Ciprofloxacin, moderately sensitive to Cefalexin and Amoxicillin, and resistant to Nalidixic acid and Erythromycin which supports the findings of Nazir et al, (2004).

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TABLES

Table 1. Bacteria isolated from different organs of 3 batches of DOC.

ID No	Grade	No of chicks	Sample type	Name of isolated bacteria
	A	2	Gut	*E. coli*
			Liver	-
			Lungs	-
Batch 1	B	2	Gut	*E. coli*
			Liver	-
			Lungs	-
	C	2	Gut	*E. coli*
			Liver	*E. coli*
			Lungs	*E. coli*
	A	2	Gut	*E. coli*
			Liver	-
			Lungs	-
Batch 2	B	2	Gut	*E. coli*
			Liver	*E. coli*
			Lungs	-
	C	2	Gut	*E. coli*
			Liver	*E. coli*
			Lungs	*E. coli*
	A	2	Gut	*E. coli*
			Liver	-
			Lungs	-
Batch 3	B	2	Gut	*E. coli*
			Liver	*E. coli*
			Lungs	-
	C	2	Gut	*E. coli*
			Liver	*E. coli*
			Lungs	*E. coli*

Table 2. Total viable count (TVC) obtained from gut of DOC.

	Sample Type	**TVC (CFU/ml)**	**Mean±SD**
Gut	Grade A	2.3×10^6	2.6×10^6
	Grade B	2.6×10^6	
	Grade C	2.9×10^6	
Liver	Grade A	2.6×10^5	2.7×10^5
	Grade B	2.7×10^5	
	Grade C	2.8×10^5	
Lung	Grade A	2.2×10^4	2.3×10^4
	Grade B	2.3×10^4	
	Grade C	2.5×10^4	

Table 3. Biochemical characteristics of *E. coli*.

Tests		Results
Reaction with five basic sugars	Dextrose	+
	Maltose	+
	Lactose	+
	Sucrose	+
	Mannitol	+
Indole		+
MR		+
VP		-

+ indicates Positive, MR = Methyl Red, - indicates Negative, VP= Voges-Proskauer

Table 4. Antimicrobial agent with their disc concentration.

Name of Antibiotics	Disc concentration (µg /disc)
Amoxicillin (Amp)	10
Cephalexin (CI)	30
Enrofloxacin (ENRO)	30
Ciprofloxacin (Cip)	5
Cloxacillin (OB)	5
Erythromycin (E)	15
Kanamycin (K)	30
Nalidixic acid (NA)	30

Legend: µg = Micro gram

Table 5. Antibiotic sensitivity pattern in percentage.

Name of organisms	Sensitivity pattern	% of isolated strains sensitive to various antibiotic							
		AMO-X	ENRO	CIP	CI	E	K	NA	OB
E. coli	Resistance	11.3	12.1	0	0	33.3	0	66.7	66.7
	Less sensitive	61.1	43.7	16.3	66.7	66.7	33.3	33.3	33.3
	Moderately sensitive	27.6	22.1	50	33.3	0	66.7	0	0
	Highly sensitive	0	22.1	33.7	0	0	0	0	0

Legends

AMOX	=	*Amoxicillin*	*E*	=	*Erythromycin*
ENRO	=	*Enrofloxacin*	*K*	=	*Kenamycin*
CIP	=	*Ciprofloxacin*	*NA*	=	*Nalidaxic acid*
Cl	=	*Cephalexin*	*OB*	=	*Cloxacillin*

Table 6. Antibiotic sensitivity pattern.

Name of isolates	AMOX	ENRO	CIP	Cl	E	K	NA	OB
E. coli	-	+ + +	+ + +	+ +	-	+ +	+ +	-
	+	+	+ + +	+	-	+ +	+	+
	+	-	+ +	+	+	+	-	+
	+	+	+ +	+	+	+ +	+ +	+
	+	+ +	+	+	+	+ +	+ +	+
	+ +	+	+ +	+ +	+	+	+	+

Legends

AMOX		*Amoxicillin*	*NA*		*Nalixic acid*
ENRO	=	*Enrofloxacin*	*OB*	=	*Cloxacillin*
CIP	=	*Ciprofloxacin*	-	=	*Resistant*
Cl	=	*Cephalexin*	+	=	*Less sensitive*
E	=	*Erythromycin*	++	=	*Moderately sensitive*
K	=	*Kanamycin*	+++	=	*Highly sensitive*

FIGURES

Fig 1.

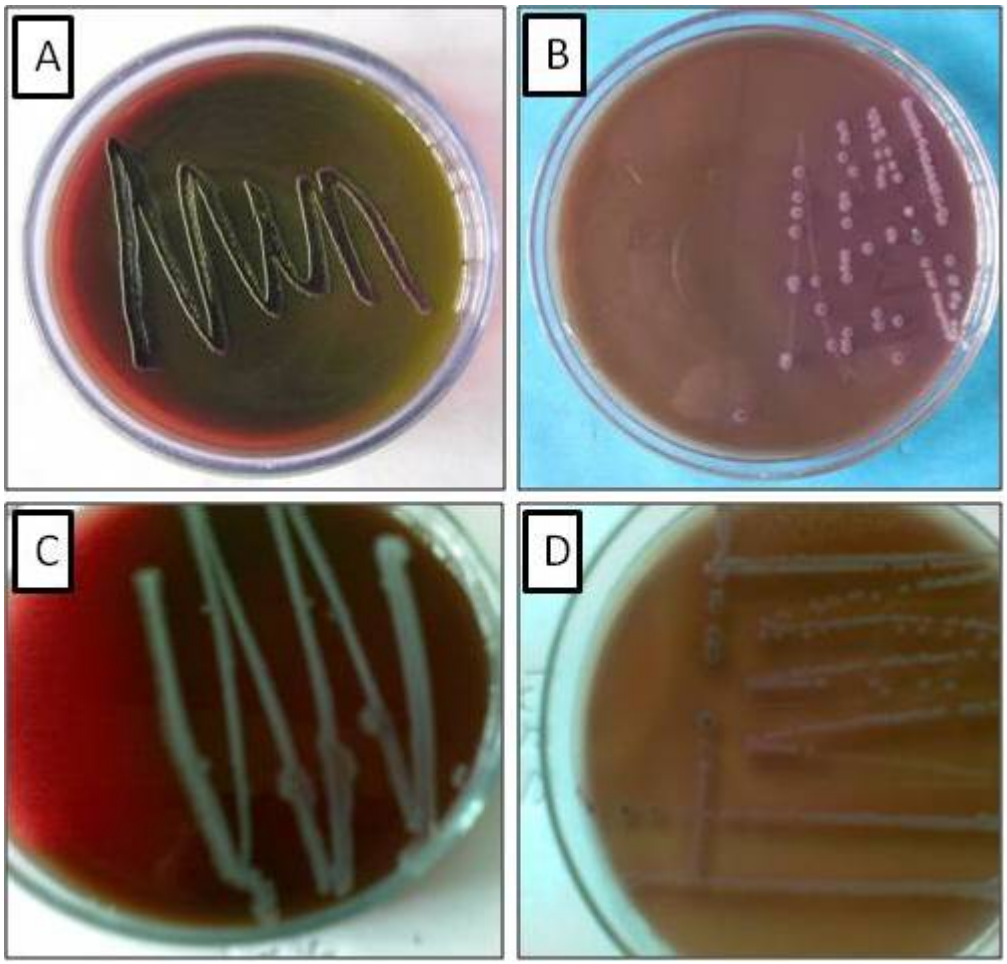


Fig 2.

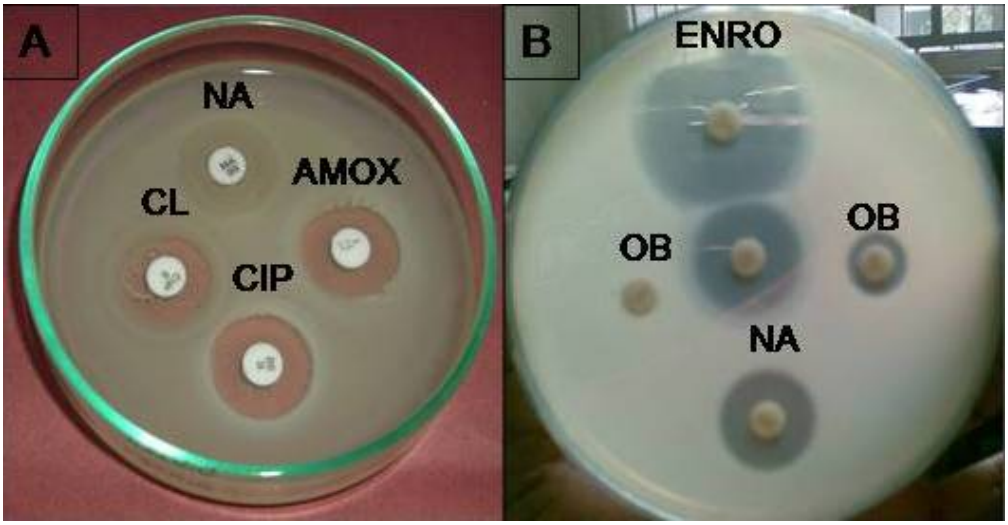
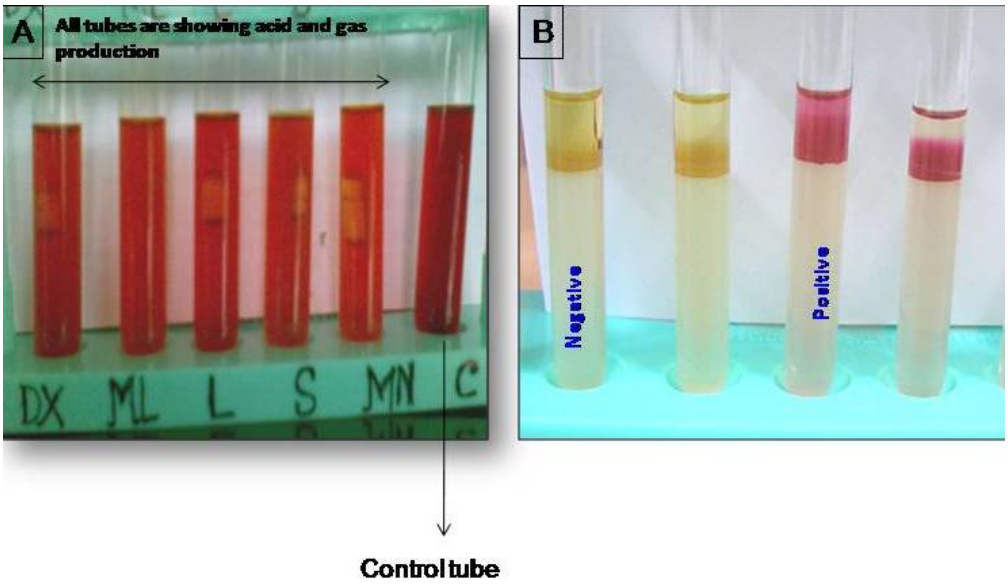


Fig 3.


A
All tubes are showing acid and gas production
DX ML L S MN C
B
Negative
Positive
Control tube