Wayamba Journal of Animal Science – ISSN: 2012-578X; P312 - P316, 2012
First Submitted April 20, 2012; Number 1336650338

ISOLATION, IDENTIFICATION AND ANTIBIOGRAM OF *ESCHERICIA COLI* FROM BROILER AT CHITTAGONG DISTRICT IN BANGLADESH

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The study was conducted to isolate and identify *Escherichia coli* from dead broiler chickens from different poultry farms in Chittagong, Bangladesh, from July, 2010 to June, 2011 and also the antibiogram of the isolates. A total number of 275 dead broiler chickens were examined and 150 (54.55%) were diagnosed as infected with *E. coli.* The *E. coli* were isolated and identified by cultural and biochemical characteristics. The isolated *E. coli* were highly sensitive to colistin sulphate followed by ciprofloxacin, amoxicillin, ampicillin, oxytetracyclin, and resistant to cotrimoxazole, gentamycin and penicillin. It may be concluded from the results of this study that the high resistance of *E. coli* to antibiotics constitutes a threat to poultry industry in Bangladesh.

Key Words: *Escherichia coli*, Broiler, Antibiogram

Escherichia coli is one of the common microbial flora of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both (Jawetz et al., 1984 and Levine, 1987). Although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10-15 % of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes and Gross, 1997) and cause a variety of lesions in immunocompromised hosts as well as in poultry. Among the diseases some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (Daini et al., 2005) and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis in poultry (Gross, 1994). During the past two decades, severe outbreaks of gastrointestinal illness have been occurred by food borne pathogenic *E. coli* (Armstrong et al., 1996).

Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Anti-microbial abuse is considered to be the most vital selecting force to antimicrobial resistance of bacteria (Moreno et al., 2000 and Okeke et al., 1999). Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992 and Witte, 1998). Therefore, the present study was designed to isolate *E. coli* strains from five different sources of poultry and poultry environment of Bangladesh for assessing their resistance patterns to some selected antimicrobials.

MATERIALS AND METHODS

Study Period and place

This study was carried out in the Department of Microbiology, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Chittagong from July, 2010 to June, 2011.

Sample collection

All samples (liver, heart, lungs and intestine) were collected from post mortem chickens in separate zipper lock bag and then it was kept in deep freeze (- 20^0C) for bacteriological examination to identify the *Escherichia coli* colony.

Transportation of sample:

After collection, all the samples were transported to the laboratory immediately in an insulating foam box with ice.

Isolation and identification of *E. coli*

For isolation of *E. coli*, samples were first inoculated on MC (Mac Conkey) agar and incubated at 37°C for 24 hours. To identify *E. coli* and other coli forms lactose fermenting red colonies from the MC agar were sub-cultured on EMB (Eosin Methylene Blue) agar. Colonies on EMB agar with metallic sheen were suspected as positive for *E. coli* and were confirmed by biochemical test (IMViC test). *E. coli* was found positive to indole test but negative to MR (Methyl Red) and VP (Vogus proskauer) test.

Antibiotic sensitivity test:

Antibiotic sensitivity test of isolated *E. coli* was performed with the standardized commercial sensitivity discs following Disc Diffusion Method (Bauer et al., 1966). A total of eight antibiotic discs (Becton Dickinson, U.S.A.) with Ciprofloxacin 5µg, Colistin sulphate10 µg, Cotrimoxazole 10µg, Tetracycline 30µg, Penicillin 10 µg, Ampicillin 10 µg, Amoxicillin 10 µg and Gentamicin 10µg were used. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2 ml of Mueller-Hinton broth. The broth culture was then allowed to incubate at 37 °C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 min and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of LB (Luria Broth) agar to obtain uniform inoculums. The plates were then allowed to dry for 3-5 min. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Within 15 min of the application of the discs, the plates were inverted and incubated at 37 °C. After 16-18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA.

RESULTS AND DISCUSSION

A total number of 275 dead broiler chickens were examined within 12 months of period. Within 275 birds, 150 (54.55%) were diagnosed as infected with *Escherichia coli* (Table-01).

Table-01: *E coli* affected broiler chicken in different region

Region	Total Post – mortem Birds	*E. coli* affected Bird	Percentage
Chittagong	36	24	66.67
Anowara	26	22	84.61
Patiya	36	26	72.22
Rawjan	46	13	28.26
Hatajari	68	39	57.35
Sitakundu	25	12	48.00
Bualkhali	13	8	61.53
Fotikchori	6	3	50.00
Chandanais	13	2	15.38
Bashkhali	4	0	0.00
Lohagara	2	0	0.00
Total	**275**	**150**	**54.55**

These results support the earlier reports of Suha et al., (2008) who reported 43.50% and reports of Rahman et al., (2004) who reported 67.73% colibacillosis in commercial broiler and layer. These results also support the earlier reports of Hossain et al., (2008) who reported 60% colibacillosis in commercial broiler and layer birds. Bhattacharjee et al., (1996) reported 40.82% and Ahmed et al., (2009) reported 52.26% prevalence of *E. coli* in chicken from Bangladesh but Nazir (2004) stated the over all prevalence was 62.5% from chicken, which is closed to the present findings.

Bacteriological media and biochemical tests were used for the isolation and identification of the bacteria which coincides with the results of Buxton and Fraser (1977), Cowan (1985), and Cheesbrough (1985), described that the isolated *E. coli* ferment five basic sugars with production of acid and gas.

Table-02: Antibiotic sensitivity pattern in percentage

Name of organisms	Sensitivity pattern	% of isolated strains sensitive to various antibiotic							
		CN	CT	CIP	AMP	AML	P	SxT	OT
E. coli	Resistance	75.00	0.00	0.00	0.00	25.00	75.00	100.00	50.00
	Less sensitive	0.00	0.00	50.00	100.00	75.00	25.00	0.00	50.00
	Moderately sensitive	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00
	Highly sensitive	25.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00

CN=Gentamycin, AML=Amoxicillin, P=Penicillin, SxT=Cotrimoxazole, OT=Oxytetracyclin, CT=Colistin sulphate, CIP=Ciprofloxacin, AMP=Ampicillin

Antibiotic sensitivity pattern of isolated *E. coli* was performed after isolation against eight commonly used antibiotics belonging to different groups (Table-02). From the antibiotic sensitivity study it was observed that *E. coli* were highly sensitive to colistin sulphate (100%), moderately sensitive to ciprofloxacin (50%) and these findings were almost similar to the reports of Nazir et al.,(2005). Less sensitive was to amoxacillin (100%), ampicillin (75%), oxytetracyclin (50%) and penicillin (25%) and resistant to cotrimoxazole (100%), gentamycin (75%), penicillin (75%) and these findings almost similar to the report of Okoli et al., (2006). Rahman et al., (2004) reported *E. coli* was highly sensitive to ciprofloxacin. They state that it was might be due to fact that it has been recently introduced, have broad spectrum of action and limit used so far by the poultry farmers. So, it may be concluded that the frequency of *E. coli* from dead broiler chickens and the antibiogram nature of the isolates is quite significant in respect of indiscriminate use of antibacterial drugs.

CONCLUSION

The study clearly suggests that that the frequency of *E. coli* from dead broiler chickens and the antibiogram nature of the isolates is quite significant in respect of indiscriminate use of antibacterial drugs.

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