Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 3 (2): 82-90, 2008
ISSN 1816-4935

Antibiogram and Plasmid Profile Analysis of Isolated *Escherichia coli* from Broiler and Layer

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Abstract: A total of 17 *Escherichia coli* isolated from 24 fresh faecal samples of broiler and layer were screened for their antibiograms and plasmid profiles. The overall recovery rate of *E. coli* from faecal samples was 70.83%. All *E. coli* strains were analyzed to determine their susceptibility patterns to 8 commonly used antibiotics (ampicillin, cephradine, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, tetracycline and sulphamethoxazole) belonging to different groups. From the antibiogram study it was revealed that 87.50% *E. coli* isolated from broiler were resistant to both ampicillin and sulphamethoxazole. Only 37.50% broiler isolates were highly sensitive to gentamicin and 50% isolates to chloramphenicol. All the *E. coli* isolates of layer were completely resistant (100%) to sulphamethoxazole and about fifty 5% of the isolates (55.55%) were resistant to both streptomycin and tetracycline. *E. coli* isolated from layer were found to be highly sensitive (44.44%) to chloramphenicol and 66.66% were also highly sensitive to gentamicin. Plasmid profile of 17 isolates was analyzed by 0.8% agarose gel electrophoresis. A total of 8 different plasmid bands of different size were estimated by eye comparing to reference marker. The estimated size of the bands were 3.25, 5.20, 6.00, 8.00, 15.0, 30.0, 33.5 and 38.0 kbp. Plasmid profile analysis of the isolated *E. coli* revealed that the isolates carrying multiple plasmids which might be the cause of various degrees of antibiotic resistant. The plasmids were distributed at random in the isolated *E. coli* strains and there was no remarkable interrelationship between antibiotic resistance and plasmid present. In most of the cases, strains having similar plasmid bands but confer resistant to different antibiotics. In some cases, isolates showed resistance to antibiotics but did not harbor any plasmid indicating that chromosomal DNA may carry the genes that confer resistance to antibiotics.

Key words: *Escherichia coli*, antibiogram, plasmid profile, broiler, layer

INTRODUCTION

Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). This acquired resistance occurs not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (Hinton *et al*., 1982; Piddock, 1996; Van den Bogaard, 1997; Van den Bogard and Stobberingh, 1999). In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals and antimicrobial agents may be continuously fed to food animals such as broilers and layers as

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antimicrobial growth promoters. Therefore the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria (Caudry and Stanisch, 1979).

At slaughter, resistant strains from the gut may contaminate poultry carcasses and as a result poultry meats are often associated with multiresistant *Escherichia coli* (Chaslus-Dancla and Lafont, 1985; Jayaratne *et al*., 1990; Turtura *et al*., 1990); likewise eggs become contaminated during laying (Lakhotia and Stephens, 1973). Hence, antimicrobial resistant faecal *E. coli* from poultry can infect humans both directly and via food. Although rare, these resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora.

It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Hassan, 1995). This therefore demands the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities. According to Aibinu *et al*. (2004) *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim-sulfamethoxazole. The widespread occurrence of drug resistant *E. coli* and other pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both (Omigie *et al*., 2006).

There is strong evidence that the use of antimicrobial agents can lead to the emergence and dissemination of resistant *E. coli* (Van den Bogaard *et al*., 2001; Galland *et al*., 2001; Schroeder *et al*., 2002), which can then be passed onto people via food or through direct contact with animals. The finding of multiple drug resistant commensal *E. coli* isolates from broiler chickens from farms that did not report antibiotic use concurs with these latter reports. Sayah *et al*. (2005) reported that farm environmental isolates showed reduced susceptibility (as measured by disc diffusion zone sizes) compared to faecal sample isolates to most agents studied. They suggested that non-sampled sources, e.g., farm workers and wildlife with access to the farm environment, could be sources of resistance factors.

E. coli is a member of the family *Enterobacteriaceae*, which includes many genera, including known pathogens such as *Salmonella*, *Shigella* and *Yersinia*. Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens that cause infection in immunocompromised hosts. Most strains of *E. coli* live as commensals, many perhaps all are opportunistic pathogens of human and animals (Levine, 1983). In poultry especially in broiler of 6-9 weeks of age Colibacillosis is a major disease caused by *E. coli* (Buxton and Fraser, 1977). Colibacillosis is a major disease in poultry of 0-8 weaks of age in Bangladesh (Saleque, 2003). During recent year the wide spread use of antibiotics in the field of veterinary medicine have resulted in the development of increasing number of bacterial strains possessing resistance to many antibiotics. The property of multidrug resistance could be transferred through conjugation from resistant strains of *E. coli* to another by means of plasmid, which occur in cytoplasm of the donor bacterium and multiply independently of the chromosomal DNA. Thus a new bacterium with resistance factor emerges that is resistant to one or more antimicrobial agents (Buxton and Fraser, 1977). *E. coli* exists in large numbers in the intestinal flora, which indicates tremendous potential for plasmid dissemination in nature (Freeman *et al*., 1985). So the present study was conducted to find out the correlation between antibiotic sensitivity pattern and plasmid profile of the poultry *E. coli* isolates and also to find out the effective antibiotic(s) against *E. coli*.

MATERIALS AND METHODS

This study was conducted during the period of July to December' 2005 in the Department of Microbiology and Hygiene and in the Central laboratory, Bangladesh Agricultural University.

Sample Collection

A total of 24 fresh faecal samples were collected randomly from four poultry farms adjacent to Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. All samples 100 g were collected in sterile containers. Faecal samples were collected with the help of sterile cotton bud and transferring the buds immediately to sterile nutrient broth in sterile screw capped test tubes. At each time of collection, precaution was taken to prevent or avoid cross-contamination of samples. After collection of the samples, they were transported to the laboratory as soon as possible in an insulated foam box with ice to maintain a temperature ranging from -4 to 6°C and bacteriological analyses were performed within 4 h of collection.

Isolation of *E. coli* in Pure Culture

Primary culture was done in nutrient broth and then pure cultures were obtained using McConkey agar and Eosine Methylene Blue (EMB) agars. The pour-plate technique was followed to get the pure culture of *E. coli*. The colony characters were observed and staining was performed by Gram's methods. Isolates yielding similar biochemical tests (indole test, Methyl Red (MR) test, Veges Proskauer (VP) test, citrate test, catalase test and sugar fermentation) to the standard *E. coli* strain, ATCC 25922 were identified as *E. coli* and selected for further testing.

Determination of Antimicrobial Sensitivity Pattern

Sensitivity of *E. coli* isolates to different antimicrobial agents was determined *in vitro* by employing a modified disk diffusion test of the Kirby-Bauer (Bauer *et al*., 1966) method. Antibiotics used in this study are ampicillin, cephradine, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, tetracycline and sulphamethoxazole. Cartridges of antimicrobial-containing discs were obtained from Mast Diagnostics (Merseyside, UK), stored between 4 and 20°C and allowed to come to room temperature prior to use. Isolates were subcultured from the bank onto Miller's LB agar and incubated for 18-24 h before being transferred to 5 mL sterile 0.9% saline to match the 0.5 MacFarland standard (Remel, Kansas). A sterile cotton-tipped swab was used to streak air-dried Mueller-Hinton II plates within 15 min of adjustment of turbidity. Subsequently, antimicrobial discs were added and plates were incubated aerobically at 35±2°C for 16-18 h. The diameter of the zones of inhibition surrounding the antimicrobial discs was measured to the nearest mm. Isolates were deemed resistant only when the zone of inhibition was less than or equal to the resistance breakpoint recommended by the guidelines of NCCLS (2002). Quality control was performed as recommended using *E. coli* strain ATCC 25922.

Plasmid DNA Extraction, Reference Marker and Agarose Gel Electrophoresis

Smaller circular plasmid DNA molecules were extracted from the huge chromosomal DNA for analysis on DNA. The selected bacterial strain (single colony) was grown overnight in LB broth at 37°C with aeration using the orbital shaker. The 1.5 mL overnight culture was taken in eppendorf tube for plasmid DNA extraction. The plasmid DNA from *E. coli* isolates was extracted through Mini alkaline lysis by SDS (Sambrook and Russel, 2001). The plasmid DNA extracted from *E. coli* isolates were compared to known molecular weight standards (Super mix DNA ladder, ranging from 0.5 to 33.5 kbs, Bangalore Genei Pvt. Ltd., India). Electrophoresis was carried out in a horizontal gel apparatus (Max submarine, Agarose gel unit, Model He 99). The method followed for agarose gel electrophoresis was as described by Maniatis *et al*. (1983).

RESULTS AND DISCUSSION

The overall prevalence of *E. coli* was 70.83%. The highest recovery was found from layer faeces (75%). Nazir (2004) observed 65% prevalence of *E. coli* in faecal samples of broiler, 60% in layer. The present findings were higher than the report of Nazir (2004). In nutrient broth turbid were found, in EMB agar black centered colony with metallic sheen was found. The greenish-black colonies with

metallic sheen on EMB agar were presumptively identified as *E. coli* (Pelczer *et al*., 1998). Also in Gram's staining under microscope the organism revealed gram-negative, pink color, small rod shaped that are characteristic features of *E. coli*. Several biochemical tests were performed for confirmation of *E. coli*. They were characterized by their ability to ferment glucose, sucrose, lactose, maltose, mannitol and sorbitol to produce gas (CO_2), positive for indole test and MR test and negative for VP and Citrate utilization test.

A total of 17 isolates of *E. coli* from boiler and layer were analyzed. The antibiotic sensitivity pattern and the percentage of isolates resistance, less sensitive, moderately sensitive and highly sensitive to each antibiotic are outlined in Table 1 and 2, respectively. Resistance of 75% broiler isolates to ciprofloxacin, streptomycin and tetracycline were significantly higher than those isolated from layer (33.33, 55.55 and 55.55%), whereas both layer and broiler *E. coli* isolates were 87.4% resistant to streptomycin as observed by Al-Ghamdi *et al*. (2001). Layers isolates (33.33%) were resistant to ciprofloxacin which may be due to long term and continuous use of this antibiotic and slow growth of antibiotic resistance. This is closely related to Al-Ghamdi *et al*. (2001), found 34.7% resistant to ciprofloxacin.

Table 1: Antibiotic sensitivity patterns of *E. coli* isolates of different sources

Isolates	Resistance	Less sensitive	Moderately sensitive	Highly sensitive
BR-1	AMP, S	CIP, CE, SXT	TE	C, GN
BR-4	AMP, CIP, S, SXT, TE	CE, GN	-	C
BR-5	AMP, C, CIP, S, SXT, TE	CE, GN	-	-
BR-6	AMP, CIP, S, SXT, TE	C, CE	-	GN
BR-8	AMP, C, CIP, S, SXT, TE	CE, GN	-	-
BR-9	AMP, C, CIP, SXT, TE	CE, S	GN	-
BR-10	CIP, S, SXT, TE	CE	AMP	C, GN
BR-12	AMP, SXT	-	CE, GN, S	C, CIP, TE
LY-1	AMP, C, CIP, CE, S, SXT, TE	-	GN	-
LY-2	S, SXT	CE, TE	AMP, CIP	C, GN
LY-3	AMP, SXT	CE, S	C, CIP	GN, TE
LY-4	S, SXT	TE	CE, AMP	C, GN, CIP,
LY-6	SXT, TE	C	AMP, CE, S	CIP, GN
LY-7	CIP, S, SXT, TE	CE	AMP	C, GN
LY-8	AMP, SXT	TE, S	C, CE	GN, CIP
LY-10	CE, SXT, TE	AMP, S	GN, CIP	C
LY-11	AMP, CIP, CE, S, SXT, TE	C	GN	-

AMP = Ampicillin; C = Chloramphenicol; CE = Cephradine; CIP = Ciprofloxacin; S = Streptomycin; SXT = Sulphamethoxazole; GN = Gentamicin; TE = Tetracycline

Table 2: Antibiotic sensitivity pattern in percentage

Sources of	Resistance		Less sensitive		Moderate sensitive		Highly sensitive	
E. coli isolation	Antibiotic	%	Antibiotic	%	Antibiotic	%	Antibiotic	%
Broiler	AMP	87.50	-	-	AMP	12.50	-	-
	C	37.50	C	12.50	-	-	C	50.00
	CIP	75.00	CIP	12.50	-	-	CIP	12.50
	S	75.00	S	12.50	S	12.50	-	-
	-	-	CE	87.50	CE	12.50	-	-
	TE	75.00	-	-	TE	12.50	TE	12.50
	-	-	GN	37.50	GN	25.00	GN	37.50
	SXT	87.50	SXT	12.50	-	-	-	-
Layer	AMP	44.44	AMP	11.11	AMP	44.44	-	-
	C	11.11	C	22.22	C	22.22	C	44.44
	CIP	33.33	-	-	CIP	33.33	CIP	33.33
	CE	33.33	CE	33.33	CE	33.33	-	-
	S	55.55	S	33.33	S	11.11	-	-
	SXT	100.00	-	-	-	-	-	-
	TE	55.55	TE	33.33	-	-	TE	11.11
	-	-	-	-	GN	33.33	GN	66.66

AMP = Ampicillin; C = Chloramphenicol; CE = Cephradine; CIP = Ciprofloxacin; S = Streptomycin; SXT = Sulphamethoxazole; GN = Gentamicin; TE = Tetracycline

E. coli isolates of broiler showed no resistance to gentamicin and this is closely related to Tricia *et al.* (2006). The broiler isolates highly sensitive to chloramphenicol were 50%; 12.50% of the rest isolates were moderately sensitive and the rest 37.50% were resistance to the same antibiotic. On the other hand, 12.50% isolates were highly sensitive to both ciprofloxacin and tetracycline. The isolates from broiler were not resistant to cephradine.

The isolates from layer were not resistant to gentamycin. About 66.66% of the isolates were moderately sensitive to gentamicin and the rest (about 33.33%) were highly sensitive to that antibiotic.

Most of the isolates from the faeces of broiler and layer were resistance to the antibiotics, sulphamethoxazole that was 87.50 and 100%, respectively. So the multidrug resistant *E. coli* is continuously increasing which were also reported by Hussain *et al.* (1982) and Nazir (2004). Therefore, if the current practice of the use of antibiotics is sustained, emergence of resistant *E. coli* strains to these useful drugs will follow and soon a grave situation will arise when there will be no drug available to treat life-threatening infections caused by resistant *E. coli* strains.

Among the broiler isolates 87.50% were resistant to ampicillin and 44.44% layer isolates were resistant to the same antibiotic. On the other hand, Al-Ghamdi *et al.* (2001) found 81.6% and Tricia *et al.* (2006) found 42.9% resistant to ampicillin.

Plasmid from 17 *E. coli* isolates were extracted according to the procedure described in materials and methods and analyzed by agarose gel electrophoresis. Gel electrophoresis showed 14 plasmid bands producing all together 8 different plasmid patterns. Faruque *et al.* (1992) found 25 different plasmid bands by analysis of 63 isolates. In the plasmid profile it was observed that out of 8 isolates from broiler 5 isolates showed plasmid bands. Among them 2 isolates (BR-5 and BR-9) contain 2 plasmid bands in each, 3 isolates contain 1 plasmid band in each and 3 isolates (BR-4, BR-6 and BR-12) did not contain any plasmid bands. The estimated size of the plasmid bands of BR-1, BR-5, BR-8, BR-9 and BR-10 were (38.0); (33.50, 6.0); (38.0); (38.0, 6.0) and (15.0) kbp, respectively (Table 3, Fig. 1).

Among the isolates (9) of layers 2 isolates (LY-2 and LY-6) contain two plasmid bands in each which were estimated as (33.50, 8.0) and (33.50, 5.20) kbp, respectively. One plasmid band is recorded in another three isolates (LY-4, LY-8, LY-10). Their estimated sizes were recorded as 3.25, 30.0 and 15.0. Rest of the isolates (LY-1, LY-3, LY-7 and LY-11) did not harbor any plasmid band (Table 3, Fig. 2).

Table 3: Plasmid profile and antibiotic sensitivity patterns of *E. coli*

Isolates	Resistance to antibiotics	No. of plasmid bands	Plasmid size in kbp
BR-1	AMP, S	1	38.0
BR-4	AMP, CIP, S, SXT, TE	0	-
BR-5	AMP, C, CIP, S, SXT, TE	2	33.50, 6.0
BR-6	AMP, CIP, S, SXT, TE	0	-
BR-8	AMP, C, CIP, S, SXT, TE	1	38.0
BR-9	AMP, C, CIP, SXT, TE	2	38.0, 6.0
BR-10	CIP, S, SXT, TE	1	15.0
BR-12	AMP, SXT	0	-
LY-1	AMP, C, CIP, CE, S, SXT, TE	0	-
LY-2	S, SXT	2	33.50, 8.0
LY-3	AMP, SXT	0	-
LY-4	S, SXT	1	3.25
LY-6	SXT, TE	2	33.50, 5.20
LY-7	CIP, S, SXT, TE	0	-
LY-8	AMP, SXT	1	30.0
LY-10	CE, SXT, TE	1	15.0
LY-11	AMP, CIP, CE, S, SXT, TE	0	-

AMP = Ampicillin; C = Chloramphenicol; CE = Cephradine; CIP = Ciprofloxacin; S = Streptomycin; SXT = Sulphamethoxazole; GN = Gentamicin; TE = Tetracycline

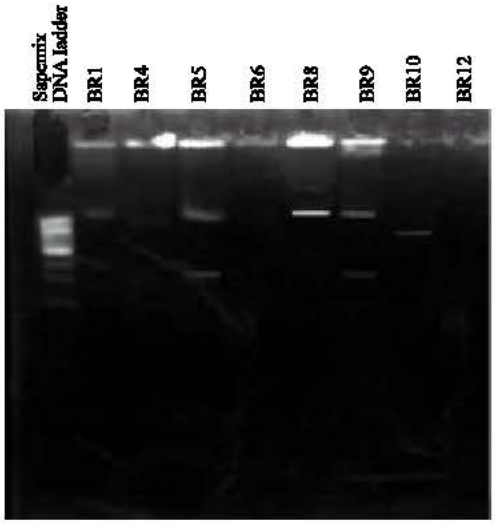

Fig. 1: Plasmid profile of *E. coli* isolates from broiler faecal sample analyzed by 0.8% agarose gel electrophoresis after staining with ethidium bromide and DNA bands were visualized by UV-transilluminator and took photograph by Gel Documentation

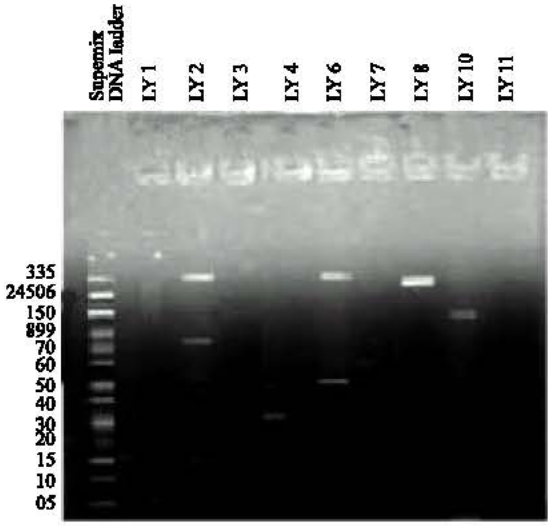

Fig. 2: Plasmid profile of *E. coli* isolates from layer faecal sample analyzed by 0.8% agarose gel electrophoresis after staining with ethidium bromide and DNA bands were visualized by UV-transilluminator and took photograph by Gel Documentation

The plasmids were distributed at random in the isolated *E. coli* strains and there was no remarkable interrelationship between plasmid patterns and antibiotic resistance patterns. In most of the cases, strains having similar antibiotic sensitivity patterns had different plasmid bands like isolates BR-5 and BR-8. In some cases, isolates showed resistance to antibiotics but did not harbor any plasmid (BR-4, BR-6, BR-12 and LY-1, LY-3, LY-7, LY-11) indicating that chromosomal DNA may carry the genes that confer resistance to antibiotics.

From the study, it was found that most of the isolates were resistant to five antibiotics such as ampicillin, ciprofloxacin, streptomycin, sulphamethoxazole and tetracycline. Such high incidence of multidrug resistance may presumably be due to indiscriminate use of antibiotics at the present time, which may eventually supercede the drug sensitive microorganisms from antibiotic saturated environment (Jawetz *et al*., 1984). From the plasmid profile analysis it was revealed that some isolates carried multiple plasmids, some carried single plasmid and some carrying no plasmids which correlates with the results of Lee *et al.* (2000). Through the discharges of human, animal and bird faecal materials, drug resistant bacteria are distributed in the sewage and surface water where exchange of R-plasmids can occur under certain physico-chemical and biological conditions (Anonymous, 1978). The drug resistant bacteria can spread in the environment where man and animal acquire infection with bacteria carrying drug resistant plasmids (Joseph *et al.*, 1979).

The plasmids were distributed at random in the isolated *E. coli* strains. *E. coli* isolates contained single or multiple plasmids bands and showed multiple drug resistance patterns. These multiple drug resistance patterns of the *E. coli* isolates of this study might be due to drug resistance gene (s) carried out by the different plasmids (Freeman, 1985; Bakshi *et al*., 2003). In most of the cases, strains having similar antibiotic sensitivity patterns but showed different plasmid patterns. An isolate like BR-4 showed resistance to 5 antibiotics but did not harbor any plasmid. This supposition has been supported by the finding that the plasmidless strains may also be resistant to one or more antibiotics. Therefore, there was no noticeable correlation between antibiotic resistance patterns and plasmid patterns.

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