Bangl. J. Vet. Med. (2008). 6 (1): 13–18

DRUG SENSITIVITY PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM SAMPLES OF DIFFERENT BIOLOGICAL AND ENVIRONMENTAL SOURCES

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ABSTRACT

A total of 100 different *E. coli* isolates collected from 10 different biological and environmental sources (10 isolates from each source) such as human faces, human urine, cattle, sheep, goat, chicken, duck, pigeon, drain sewage and soil were used for in-vitro drug sensitivity test in the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh during the period from January to May 2007. Ten different drugs such as Gentamicin (GM), Azithromycin (AZM), Erythromycin (E), Levofloxacin (LVX), Ciprofloxacin (CIP), Tetracycline (TE), Amoxicillin (A), Ampicillin (AP), Nalidixic acid (NA) and Metronidazole (MET) were used in this study. Sensitivity test was carried out by the Kirby-Bauer disc diffusion method as per recommendation of National Committee for Clinical Laboratory Standards and efficacy of a drug was determined by measuring the diameter of the zone of inhibition that results from diffusion of the agent in to the medium surrounding the disc. A high of 80% and 78% *E. coli* isolates collectively from all the selected sources were sensitive to LVX and CIP respectively, followed by GM (46%), AZM (45%), TE (30%), AP (29%), E (19%), NA (18%) and A (15%). No isolate was sensitive to MET (0%). Incase of resistance, 96% isolates were resistant to MET, followed by A (72%), E (69%), NA (67%), TE (60%), AP (59%), AZM (33%) and GM (32%), CIP (8%) and LVX (5%). A number of isolates showed intermediate reaction to GM (22%), AZM (22%), LVX (15%), NA (15%), CIP (14%), A(13%), AP (12%), E (12%), TE (10%) and MET (4%). This may be an intermediate phase for the conversion of *E. coli* isolates from sensitive to resistant form. From the research it may be concluded that *E. coli* infection of different animals and birds and also of human being may be treated effectively with LVX and CIP followed by GM and AZM.

Key words: *E. coli* isolates, levofloxacin, ciprofloxacin, efficacy, resistance

INTRODUCTION

E. coli is a major pathogen of commercially produced poultry causing colibacillosis all over the world. In birds, it causes airsacculitis, pericarditis, septicemia, and death (Hofstad *et al*., 1984). It is a major cause of respiratory and septicemic diseases in broiler chicken causing mortality less than 5% and morbidity over 50% and also affects layers resulting failure of productivity and fertility of eggs (Barens and Gross, 1997). It may cause about 28% death in Sonali birds (Biswas *et al.*, 2006). The prevalence of *E. coli* infection is higher in diarrhoeic calves about 13.71% compared to non-diarrhoeic calves about 9.1% and the overall prevalence of *E. coli* incase of colibacillosis affecting calves is 97.38% and 28% of the total death in calves occurred in first month of life and 50% of death during first week due to *E. coli* infection (Debnath *et al.*, 1990). Colisepticemia also occurs in sheep causing mortality ranged from 1% to 5% with an age distribution of 3 to 12 weeks old (Mason and Corbould, 1981). *E. coli* is the most common cause of food and water-borne human diarrhea worldwide in developing countries causing 800000 deaths out of 650 million cases per year primarily in children under the age of five years (Turner *et al.*, 2006). It also causes urinary tract infection and other complications in human. *E. coli* is an important zoonotic pathogen. *E. coli* O157:H7 is pathogenic for human but non-pathogenic in cattle and present in the feces of healthy cattle (Elder *et al.*, 2000).

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Goats, sheep, and swine can also be carriers of *E. coli* O157:H7. Because of the lack of an efficient commercial vaccine, the control of colibacillosis mainly relies on the use of antimicrobial drugs. However, bacteria have developed strategies for survival within the host during an infection and one of these strategies is the resistance of isolates to the antimicrobial drugs. Antibiotic resistance is a serious problem because it limits the therapeutic possibilities in the treatment of bacterial diseases in domestic animal species in general and poultry in particular (Nicole *et al*., 2000). According to Hussain *et al.,* 1982, the number of multi-drug resistant *E. coli* are continuously increasing although various antimicrobial agents are being used.

Considering the above facts, the present research work was undertaken to determine the current status of drug sensitivity and resistance pattern of the *E. coli* isolated from 10 different sources to select the drugs of choice for therapeutic use against various infections of man and animals caused by the organism.

MATERIALS AND METHODS

E. coli isolates

Hundred isolates of *E. coli* were used in this study which were previously isolated and identified, from 10 different biological and environmental sources (10 isolates from each source) such as human feces, human urine rectal swab of cattle, sheep and goat, cloacal swab of chicken, duck and pigeon, Drain sewage and soil (Zinnah *et al.,* 2007).

Antimicrobial discs

Commercially available antimicrobial discs (BENEX Limited, USA) were used to determine the drug sensitivity and resistance pattern of the *E. coli* isolates. A number of 10 different drugs with different disc concentration such as gentamycin (GM) 10 µg/disc, azithromycin (AZM) 15 µg/disc, erythromycin (E) 15 µg/disc, levofloxacin (LVX) 5 µg/disc, ciprofloxacin (CIP) 5 µg/disc, tetracycline (TE) 30 µg/disc, amoxicillin (A) 10 µg/disc, ampicillin (AP) 10 µg/disc, nalidixic acid (NA) 30 µg/disc and metronidazole (MET) 80 µg/disc were used in this study.

Antimicrobial sensitivity test of E. coli isolates

The antimicrobial sensitivity test of each isolate was carried out by the Kirby-Bauser disc diffusion method (Bauser *et al*., 1966) as per recommendation of National Committee for Clinical Laboratory Standards (NCCLS, 1997). This method allowed for rapid determination of invitro efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc.

The suspension of each test isolate was prepared in nutrient broth by overnight culture. By sterile syringe 0.2 ml of broth culture of the test isolate was poured on EMB agar and nutrient agar separately. Sterile glass spreader was used to spread the culture homogenously on the medium. Inoculated plates were allowed to dry for approximately 3-5 minutes and then the antibiotic discs were applied aseptically to the surface of the inoculated agar with the help of a sterile forceps. The plates were then inverted and incubated at 37ºC for 24 hours.

After incubation the plates were examined and the diameter of the zone of complete inhibition was measured by millimitre scale. The zone diameters for individual antimicrobial agent were translated into sensitive, intermediate and resistant categories.

RESULTS AND DISCUSSION

The sensitivity and resistance pattern of different *E. coli* isolates to different drugs are presented in Table 1. The current study revealed that high percentage of *E. coli* isolates from human urine were sensitive to GM (80%) and a few percent was sensitive to LVX (40%) and AZM (20%) but resistant to CIP, AP, NA and other drugs used. These findings were in close agreement with Lazarevic *et al.* (1998) and Gulsun *et al*. (2005) in term of GM which was 90-100% and 72% sensitive shown by them, respectively. At the same time, the present findings were contradictory with them in terms of CIP, AP, NA which were 85%, 35% and 90-100% sensitive respectively. Present findings also differed with the report of Sanchez Merino *et al.* (2003). They showed 77.1-81.6% sensitivity to CIP and 41.4-44% sensitivity to AP.

In the present study, all the *E. coli* isolates from human feces found to be sensitive to GM (100%), CIP (100%) followed by LVX (90%), TE (90%), AP (90%) and A (60%) showing no marked resistance to any drug except MET (90%) which were contradictory with the findings of Shehabi *et al*. (2006).

They showed that the isolates were 67%, 63%, 32% and 33% resistant to AP, NA, GM and TE, respectively. Present study was also contradictory with the report of Macias *et al*. (2002). They showed that the resistance to TE and AP was 64.4% and 52.63%, respectively.

In case of cattle high percentage of *E. coli* isolates were sensitive to LVX (80%) and CIP (80%); a few number of isolates were sensitive to AZM (30%) and NA (30%) and resistant to TE (80%), AP (90%), E (90%), A(90%) and MET (100%). Whereas, Joshi *et al*. (1986) reported that high percentage of isolates were sensitive to TE (90.90%) and GM (54.54%) and resistant to AP (36.36%) and E (27.27%), Jordan *et al.* (2005) showed resistance to TE (3.6%), A (2.2%) and GM (0.09%), Orden *et al*. (2000) showed resistance to TE (above 65%), AP (23 - 50%) and sensitive to GM (89-95%) and Sawant *et al*. (2007) found resistance to AP (48%) and TE (93%).

Current study showed that high percentage of sheep isolates were highly sensitive to LVX (90%) and CIP (90%); a moderate number to AZM (60%) and a few to A (40%) and resistant to TE (80%), AP (90%), E (90%), NA (90%) and MET (100%). *E. coli* isolates from goat were sensitive to all the drugs except MET; being cent percent sensitive to LVX and CIP followed by TE (90%), AP (90%) and NA (50%) but resistant to E (70%), AZM (60%), GM (50%), A (50%) and MET (100%). In both cases the findings were in partial agreement with Cid *et al*. (1996), who reported that isolates from lambs and kids were above 70% resistant to TE and 30-50% resistant to AP but were highly sensitive to CIP.

Table 1. Demonstration of the sensitivity and resistance pattern of different *E. coli* isolates to different drugs

Sources of *E.coli* (n = 10)		Sensitivity and resistance pattern of different *E. coli* isolates to different drugs														
		GM			AZM			LVX			TE			AP		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Human (urine)	No.	8	1	1	2	3	5	4	2	4	1	1	8	1	3	6
	%	80	10	10	20	30	50	40	20	40	10	10	80	10	30	60
Human (feces)	No.	10	0	0	8	2	0	9	1	0	9	1	0	9	1	0
	%	100	0	0	80	20	0	90	10	0	90	10	0	90	10	0
Cattle (rectal swab)	No.	4	0	6	3	2	5	8	2	0	2	0	8	1	0	9
	%	40	0	60	30	20	50	80	20	0	20	0	80	10	0	90
Sheep (rectal swab)	No.	3	1	6	6	1	3	9	1	0	1	1	8	0	1	9
	%	30	10	60	60	10	30	90	10	0	10	10	80	0	10	90
Goat (rectal swab)	No.	4	1	5	3	1	6	10	0	0	9	1	0	9	1	0
	%	40	10	50	30	10	60	100	0	0	90	10	0	90	10	0
Chicken (cloacal swab)	No.	3	1	6	0	2	8	8	2	0	0	2	8	0	1	9
	%	30	10	60	0	20	80	80	20	0	0	20	80	0	10	90
Duck (cloacal swab)	No.	2	6	2	5	3	2	7	3	0	2	1	7	0	1	9
	%	20	60	20	50	30	20	70	30	0	20	10	70	0	10	90
Pigeon (cloacal swab)	No.	6	1	3	5	3	2	9	1	0	0	2	8	0	3	7
	%	60	10	30	50	30	20	90	10	0	0	20	80	0	30	70
Drain sewage	No.	2	6	2	7	2	1	10	0	0	4	0	6	9	1	0
	%	20	60	20	70	20	10	100	0	0	40	0	60	90	10	0
Soil	No.	4	5	1	6	3	1	6	3	1	2	1	7	0	0	10
	%	40	50	10	60	30	10	60	30	10	20	10	70	0	0	10

Legends: GM = Gentamicin; AZM = Azithromycin; LVX = Levofloxacin; TE = Tetracycline; AP = Ampicillin; S = sensitive; I = intermediate; R = resistant; n = number of isolates of *E. coli* from each source.

Table 1. Demonstration of the sensitivity and resistance pattern of different *E. coli* isolates to different drugs (continued)

Sources of *E.coli* (n = 10)		Sensitivity and resistance pattern of different *E. coli* isolates to different drugs														
		CIP			E			A			NA			MET		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Human	No.	2	2	6	0	0	10	0	0	10	0	0	10	0	0	10
(urine)	%	20	20	60	0	0	100	0	0	100	0	0	100	0	0	100
Human	No.	10	0	0	6	2	2	4	2	4	3	2	5	0	4	6
(feces)	%	100	0	0	60	20	20	40	20	40	30	20	50	0	40	60
Cattle	No.	8	2	0	1	0	9	0	2	8	3	1	6	0	0	10
(rectal swab)	%	80	20	0	10	0	90	0	20	80	30	10	60	0	0	100
Sheep	No.	9	1	0	0	1	9	4	2	4	0	1	9	0	0	10
(rectal swab)	%	90	10	0	0	10	90	40	20	40	0	10	90	0	0	100
Goat	No.	10	0	0	2	1	7	4	1	5	5	2	3	0	0	10
(rectal swab)	%	100	0	0	20	10	70	40	10	50	50	20	30	0	0	100
Chicken	No.	9	1	0	0	2	8	0	1	9	1	2	7	0	0	10
(cloacal swab)	%	90	10	0	0	20	80	0	10	90	10	20	70	0	0	100
Duck	No.	6	2	2	0	1	9	0	1	9	0	1	9	0	0	10
(cloacal swab)	%	60	20	20	0	10	90	0	10	90	0	10	90	0	0	100
Pigeon	No.	8	2	0	0	1	9	0	2	8	0	1	9	0	0	10
(cloacal swab)	%	80	20	0	0	10	90	0	20	80	0	10	90	0	0	100
Drain sewage	No.	10	0	0	4	1	5	3	1	6	6	3	1	0	0	10
	%	100	0	0	40	10	50	30	10	60	60	30	10	0	0	100
Soil	No.	6	4	0	6	3	1	0	1	9	0	2	8	0	0	10
	%	60	40	0	60	30	10	0	10	90	0	20	80	0	0	100

Legends: CIP = Ciprofloxacin; E = Erythromycin; A = Amoxicillin; NA = Nalidixic Acid; MET = Metronidazole; S = sensitive; I = intermediate; R = resistant; n = number of isolates of *E. coli* from each source

In case of chicken a very high percentage of *E. coli* isolates were sensitive to CIP (90%) and LVX (80%) but resistant to other drugs such as GM (60%), NA (70%), AZM (80%), TE (80%), E (80%), AP (90%), A (90%) and MET (100%) which were in partial agreement with the findings of Islam *et al*. (2004) who found 50% of the *E. coli* isolates were resistant to AP, 100% to NA and high percentage of isolates were sensitive to CIP. Present findings were also in partial agreement with Nazir *et al.* (2005). They found that the organisms were 100% resistant to NA, 92.30% to E and 61.53% to AP while 79.92% isolates were sensitive to CIP but Yang *et al*. (2004) showed that high percentage of isolates displayed resistance to NA (100%), TE (98%), AP (79%), CIP (79%), and LVX (64%).

Most of the *E. coli* isolates from duck were found to be sensitive to LVX (70%) followed by CIP (60%) and AZM (50%) but resistant to TE (70%) AP (90%), E (90%), A (90%), NA (90%) and MET (100%). However, these findings could not be compared due to unavailability of relevant literature.

A good number of pigeon isolates were sensitive to LVX (90%) and CIP (80%) followed by GM (60%) and AZM (50%) but highly resistant to other drugs such as MET (100%), NA (90%), TE (80%), A (80%), E (90%), and AP (70%). On the other hand, Sato *et al.* (1978) found 23% TE resistant *E. coli* from domestic pigeons and 21.2% from feral pigeons. Sensitivity and resistant pattern to other drugs could not be compared due to lack of relevant literature.

The present study also revealed that isolates from drain sewage were sensitive to a good number of drugs. All the isolates were sensitive to LVX (100%) and CIP (100%) followed by AP (90%), AZM (70%) and NA (60%) but resistant to MET (100%), TE (60%) and E (50%). On the other hand, moderate number of isolates from soil were sensitive to AZM (60%), LVX (60%) and E (60%) and CIP (60%) and a few to GM (40%) but resistant to MET (100%), AP (100%), NA (90%), A (90%) and TE (70%). These findings also could not be compared because of unavailability of relevant literature.

Sensitivity of the *E. coli* isolates from different sources to a particular drug was variable. Isolates irrespective of sources showed sensitivity to GM ranging from 20-100%, to AZM ranging from 0-80%, to LVX ranging from40-100%, to TE ranging from 0-90%, to AP ranging from 0-90%, to CIP ranging from 20-100%, to E ranging from 0-60%, to A ranging from 0-40%, to NA 0-60% and to MET 0%. High percentage *E. coli* isolates collectively from all the selected sources were sensitive to LVX (80%) and CIP (78%) followed by GM (46%) and AZM (45%); low percentage of isolates were sensitive to TE (30%), AP (29%), E (19%), NA (18%) and A (15%). No isolate showed sensitivity to MET (0%).

Resistance of the *E. coli* isolates from different sources to a particular drug was also variable. Isolates irrespective of sources showed resistance to GM ranging from 0-60%, to AZM ranging from 0-80%, to LVX ranging from 0-40%, to TE ranging from 0-80%, to AP ranging from 0-100%, to CIP ranging from 0-60%, to E ranging from 10-100%, to A ranging from 40-100%, to NA 10-100% and to MET 60-100%. High percentage of *E. coli* isolates collectively from all the selected sources were resistant to MET (96%) followed by A (72%), E (69%), NA (67%), TE (60%), AP (59%), AZM (33%) and GM (32%). A very low percentage of isolates were resistant to CIP (8%) and LVX (5%).

The current study also revealed that a number of *E. coli* isolates irrespective of sources showed intermediate reaction to GM (22%), AZM (22%), LVX (15%), NA (15%), CIP (14%), A(13%), AP (12%), E (12%), TE (10%) and MET (4%). This may be an intermediate phase for the conversion of *E. coli* isolates from sensitive to resistant form.

In Bangladesh there is clear evidence of abuse of antibiotics due to which emergence of multi-drug resistant *E. coli* are continuously increasing day-by-day as stated by Hussain *et al*. (1982). Based on the present study, it may be concluded that LVX and CIP will be the first drugs of choice and GM and AZM will be the second drugs choice to resist the infections caused by *E. coli* in human, cattle, sheep, goat, chicken, duck and pigeon.

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