

Antimicrobial resistance and molecular characterisation of *E. coli* from poultry in Eastern India

Purushottam Kaushik^{1*}, Anjay¹, Savita Kumari¹, Shanker Dayal² and Sunil Kumar¹

¹ Bihar Veterinary College, Bihar Animal Sciences University, Patna, Bihar 800014, India.

² Regional Centre for Eastern Region-Indian Council of Agricultural Research, Patna, Bihar 800014, India.

*Corresponding author at: Department of Veterinary Public Health & Epidemiology, Bihar Veterinary College, Bihar Animal Sciences University, Patna, Bihar 800014, India.

Tel.: 91 9431260790, e-mail: drkaushikvet@gmail.com.

Veterinaria Italiana 2018, **54** (3), 197-204. doi: 10.12834/VetIt.330.1382.2

Accepted: 09.05.2014 | Available on line: 30.09.2018

Keywords

Poultry,
E. coli,
Anti-microbial
resistance,
Molecular
characterisation,
*stx*₁,
*stx*₂.

Summary

In this study 252 poultry samples comprised of poultry meat (n = 228) and poultry eggs (n = 24) were screened for the isolation of *Escherichia coli* (*E. coli*). A total of 62 *E. coli* isolates were recovered from poultry meat. The *E. coli* isolates belonged to different serogroups based on 'O' serotyping of the isolates viz O29 (10.8%), O8 (7.7%), O40 (6.15%), O2 (4.61%), O60 (3.08%), O106 (3.08%), 42 (1.54%), O 87 (1.54%), and O1 serotypes of O1, O7, O30, O45, O59, O66, O105, O1116, O136, O141, O147, O148, O166, and O172. Sixteen (24.62%) of the isolates were UT (untypable) and 6 (9.23 %) were rough types. Molecular characterisation of the isolates was performed, targeting *stx*₁ and *stx*₂ virulence gene fragment. Out of 62 *E. coli* isolates, 10 (16.12%) were carrying virulence gene *stx*₂, whereas none of the isolate was carrying *stx*₁ gene. The *E. coli* isolates showed wide variation in resistance pattern against the antimicrobial agents that we used (9-90%). Among *E. coli* isolates, maximum resistance was observed against cefuroxime (89.1%) and penicillin (89.4%), followed by ampicillin (80.43%), vancomycin (74.1%), co-trimoxazole (73.1%), cephalothin (60.8%), ceftriaxone (28.2%), tetracycline (17.4%), gentamicin (13%), amikacin (13.04%), ofloxacin (13%), and ciprofloxacin (6.5%). A high degree of susceptibility was observed against amikacin (84.7%) and ciprofloxacin (76%) followed by gentamicin (71.73) and ofloxacin (60.86%). High multiple antibiotic resistances were observed and a total of 34 resistance patterns were identified.

Resistenza antimicrobica e caratterizzazione molecolare di *Escherichia coli* nel pollame in India orientale

Parole chiave

Pollame,
E. coli,
resistenza antimicrobica,
caratterizzazione
molecolare,
*stx*₁ gene,
*stx*₂ gene.

Riassunto

In questo studio, condotto su 228 campioni di carne e 24 di uova, sono stati rilevati dalla carne 62 isolati di *Escherichia coli* appartenenti a differenti sierogruppi basati sul sierotipo O, ovvero O29 (10.8%), O8 (7.7%), O40 (6.15%), O2 (4.61%), O60 (3.08%), O106 (3.08%), O42 (1.54%), O87 (1.54%); per ciascun sierotipo O1, O7, O30, O45, O59, O66, O105, O1116, O136, O141, O147, O148, O166, O172, ne è stato individuato uno; 16 (24,62%) sono risultati non dimostrabili e 6 (9,23%) erano di tipi approssimativi. La caratterizzazione molecolare è stata eseguita mirando ai frammenti dei geni di virulenza *stx*₁ e *stx*₂; 10 (16,12%) trasportavano il gene *stx*₂ mentre nessuno lo *stx*₁. Gli isolati di *E. coli* hanno mostrato un'ampia variazione nel pattern di resistenza contro gli agenti antimicrobici usati (9-90%). La massima resistenza è stata osservata contro cefuroxima (89,1%) e penicillina (89,4%), seguita da ampicillina (80,43%), vancomicina (74,1%), cotrimossazolo (73,1%), cefalotina (60,8%), ceftriaxone (28,2%), tetraciclina (17,4%), gentamicina (13,0%), amikacina (13,04%), ofloxacina (13%) e ciprofloxacina (6,5%). È stato invece notato un alto grado di suscettibilità nei confronti di amikacina (84,7%) e ciprofloxacina (76%), seguita da gentamicina (71,73) e ofloxacina (60,86%). Sono state riscontrate elevate resistenze antibiotiche multiple e identificato un totale di 34 pattern di resistenza.

Introduction

Escherichia coli (*E. coli*) is a commensal bacterium in humans and animals; however some strains are capable of causing intestinal diseases. This organism is commonly present in the environment and is considered an indicator of faecal contamination of food and water. On the basis of virulence factors, *E. coli* has been classified into different pathotypes: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic or shiga toxin-producing *E. coli* (EHEC/STEC). The latter is most commonly associated with outbreaks of foodborne diseases (Bandyopadhyay *et al.* 2011). The pathogenicity of EHEC/STEC is mediated mainly through Shiga toxins 1 and 2 encoded by *stx*₁ and *stx*₂ genes, respectively. Although bovines are considered to be the major carrier for EHEC/STEC, poultry may also harbour the organism and play an important role in transmission (Ferens and Hovde 2011). The *stx*₂ gene is associated with an increased risk of developing hemolytic-uremic syndrome (Boerlin *et al.* 1999). Although reports on the isolation, identification, and characterisation of EHEC/STEC in humans and animals are available in India, very few reports on the association of EHEC/STEC in poultry are available in the country (Chattopadhyay *et al.* 2001, Khan *et al.* 2002, Wani *et al.* 2004).

During the past decade, drug resistance to Enterobacteriaceae has increased worldwide. Since *E. coli* is present as gut commensal in humans and animals, it has become one of the microorganisms that are commonly resistant to antimicrobials due to the selective pressure imposed by the antimicrobial drugs used in the treatment of food animals and humans (Zhao *et al.* 2012). Moreover, in animals antimicrobial agents are not only used for therapy and the prevention of bacterial infection, but also for the promotion of growth (Van den Bogaard and Stobberingh 2000). In the poultry sector, maximum numbers of antimicrobial agents are continuously fed to animals in order to promote growth, which further imposes selective pressure on the organism leading to development of antimicrobial resistance. The emergence of antimicrobial resistance among *E. coli* of animal origin has important public health implications. Various authors report that animals are the source of drug-resistant *E. coli* in humans (Johnson and Nolan 2009, Hammerum and Heuer 2009, Overdevest *et al.* 2011). Szmolka and colleagues (Szmolka *et al.* 2012) report common multi-resistance patterns of *E. coli* from food animals and clinical cases, which suggests that the circulation of multi-drug resistant *E. coli* circulating in the food chain is significant. The purpose of this study is to determine the prevalence, antimicrobial resistance,

and molecular characterisation of *stx*₁ and *stx*₂ genes in *E. coli* isolated from poultry meat.

Materials and methods

Sample Collection

A total of 252 samples constituting of fresh poultry meat (n = 228) and eggs (n = 24) were collected randomly from different shops and market of Patna, India during September 2010 to March 2013. The samples were transported under cold conditions to the laboratory in the Department of Veterinary Public Health, Bihar Veterinary College, Patna, India, and processed within 1 hour of collection.

Isolation and biochemical characterization

Ten grams of meat sample and egg white were inoculated in MacConkey broth (HiMedia, Mumbai, India) and incubated at 37 °C for 12 hours. Samples showing acid and gas production were further inoculated on MacConkey agar plates (HiMedia, Mumbai, India) and incubated at 37 °C for 18-24 hours. Pure and characteristic pink coloured single colonies selected from MacConkey agar were subcultured on Eosin Methylene Blue (EMB) agar (HiMedia, Mumbai, India) in order to observe the metallic sheen that is characteristic of *E. coli*. The pure colonies were picked up on Nutrient Agar slants and subjected to standard morphological and biochemical tests (Edwards and Ewing 1972).

Serotyping *E. coli*

All the biochemically confirmed isolates of *E. coli* were submitted to the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, India for serotyping based on somatic (O) antigens.

Extracting *E. coli* DNA for the detection of virulence genes

The bacterial DNA was prepared as per Dutta and colleagues (Dutta *et al.* 2011), with slight modification. The isolates were inoculated into 5 ml Luria Bertani (L-B) broth and incubated at 37 °C for 18 hours. After incubation, 1 ml of the broth culture was centrifuged in a 1.5 ml microcentrifuge tube at 8,000 rpm for 10 minutes. The pellet was washed twice in sterile normal saline solution (NSS) (0.85% NaCl) then re-suspended in 400 µl of nuclease-free sterile distilled water and boiled for 10 minutes followed by immediate chilling. Cell debris was removed by centrifugation at 5,000 rpm for

5 minutes. The supernatant was used as a template DNA for polymerase chain reaction (PCR).

Detecting virulence genes by multiplex PCR

A multiplex PCR protocol was standardized using 2 sets of oligonucleotide primers targeting *stx*₁ and *stx*₂ genes (Table I). Amplification was carried out in a total volume of 25 µl containing 10 pmol each of primer, 50 µM each of dNTP, 1.5 mM MgCl₂, 1U Taq DNA polymerase, 1X PCR of buffer, and 5 µl template DNA. A negative control containing the same reaction mixture except the DNA template was included in every experiment. The reaction condition was optimised with initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, and extension at 72 °C for 1 minute. Finally, an additional extension was achieved for 5 minutes at 72 °C. Amplified products were separated by agarose gel (2% agarose in 1X Tris-borate-EDTA buffer) electrophoresis at 5v/cm for 2 hours and stained with ethidium bromide (0.5 µg/ml) (Sambrook *et al.* 1989). A standard molecular-size marker (100 bp DNA ladder) was included in each gel. We observed and photographed DNA fragments in a gel documentation system (Biorad, USA).

Antimicrobial resistance

The antimicrobial susceptibility test of isolates was determined using the agar disc diffusion method (Wayne 2002). The antibiotic discs included ampicillin (10 µg), amikacin (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), gentamicin (10 µg), ofloxacin (5 µg), penicillin-G (10 units), tetracycline (30 µg), and vancomycin (30 µg). The isolates were grown on autoclaved Mueller Hinton broth (HiMedia, India) for 18 hours at 37 °C. About 100 µl of the inoculum was spread on Mueller Hinton agar using a sterile disposable L-shaped spreader. Antibiotic discs were placed onto the plate using sterile forceps. The plates were incubated at 37 °C for 24 hours and observed for zone of inhibition. The results were interpreted as susceptible, resistant, or moderately susceptible based on the diameter of the zone of inhibition that

was produced by different isolates. We referenced the manufacturer (Hi Media) for the breakpoint diameter of the zone of inhibition for different antibiotics. Multiple-antibiotic resistance was defined as resistance to 2 or more antibiotic classes (Shekhar and Singh 2014).

Results

Bacterial isolation and identification

A total of 62 *E. coli* out of 228 meat samples was isolated, whereas all egg samples were found negative for *E. coli*. The *E. coli* isolates belonged to 24 different serogroups: O29 (10.8%), O8 (7.7%), O40 (6.15%), O2 (4.61%), O60 (3.08%), O106 (3.08%), O42 (1.54%), O87 (1.54%), and 1 serotypes each of O1, O7, O30, O45, O59, O66, O105, O116, O136, O141, O147, O148, O166, and O172. Other results indicated 16 (24.62%) isolates as UT (untypable), and 6 (9.23 %) as rough types.

The molecular characterisation of *E. coli* by multiplex PCR

Multiplex PCR targeting *stx*₁ and *stx*₂ gene fragment of *E. coli* resulted in the amplification of 349 bp amplicon for *stx*₁ and 110 bp amplicon for *stx*₂, as we expected (Figure 1). Out of 62 *E. coli* isolates, 13 (21%) isolates that carried only the *stx*₂ gene were screened. These belonged to the serogroups O29, O8, O42, and O1.

Antimicrobial resistance

The *E. coli* isolates showed a wide variation in resistance patterns (9-90%) against the antimicrobial



Figure 1. Agarose gel electrophoresis showing amplified PCR product of *stx*₁ and *stx*₂ gene. Lane M = 100 bp DNA marker, Lane1 = Positive control for *stx*₁ (349 bp amplicon) and *stx*₂ (110 bp amplicon) gene, Lane 2-11 = *E. coli* isolates from meat samples, Lane12 = Negative control.

Table I. Oligonucleotide primers used in multiplex PCR reaction.

Primer	Sequence	Product size
<i>stx</i> ₁ F	5'-CAA CAC TGG ATG ATC TCA G-3'	349 bp
<i>stx</i> ₁ R	5'-CCC CCT CAA CTG CTA ATA-3'	
<i>stx</i> ₂ F	5'-ATC AGT CGT CAC TCA CTG GT-3'	110 bp
<i>stx</i> ₂ R	5'-CTG CTG TCA CAG TGA CAA A-3'	

agents that have been used. The maximum resistances among *E. coli* isolates were observed against cefuroxime (89.1%) and penicillin (89.4%), followed by ampicillin (80.43%), vancomycin (74.1%), co-trimoxazole (73.1%), cephalothin (60.8%), ceftriaxone (28.2%), tetracycline (17.4%), gentamicin (13%), amikacin (13.04%), ofloxacin

(13%), and ciprofloxacin (6.5%). A high degree of susceptibility was observed against amikacin (84.7%) and ciprofloxacin (76%) followed by gentamicin (71.73%) and ofloxacin (60.86%) (Tables II and III). The number of multiple antibiotic resistances pattern of isolates we observed was high and a total of 34 resistance patterns were identified (Table IV).

Table II. Antimicrobial susceptibility of *E. coli* isolates.

E. coli serotypes	Cu	G	A	Ci	Ak	Ch	Cf	Co	P	Va	Of	T
UT	R	R	R	R	R	R	R	S	R	R	R	S
UT	R	R	R	R	R	R	I	S	R	I	S	I
UT	R	I	R	I	S	R	S	S	R	I	S	S
0106	I	I	R	I	I	R	S	R	R	R	S	I
059	I	I	R	R	S	R	S	S	R	I	S	S
UT	R	R	R	I	I	I	R	S	R	I	S	S
0106	R	S	R	I	S	R	S	R	R	R	S	R
UT	R	S	R	S	S	R	S	R	R	I	S	R
Rough	I	R	R	R	R	R	S	R	R	I	S	R
Rough	R	R	R	R	S	R	S	R	R	R	R	I
UT	R	S	R	I	R	R	S	R	R	R	S	S
0136	R	S	R	S	S	R	S	R	R	S	S	S
045	R	R	R	R	R	R	S	R	R	I	S	S
Rough	R	R	R	S	S	S	S	R	R	R	I	I
UT	R	R	R	R	S	S	S	R	R	R	R	I
040	S	S	R	S	S	I	S	R	R	R	I	I
040	R	S	R	S	S	R	I	S	R	R	R	I
040	R	S	R	S	S	R	I	R	R	R	I	I
UT	S	S	R	S	S	S	S	R	R	R	S	I
Rough	R	S	R	S	S	R	I	R	R	R	R	I
UT	R	I	R	S	S	R	S	S	R	R	R	I
0147	R	S	R	S	S	R	S	R	R	I	I	S
UT	R	S	R	S	S	R	S	R	R	R	I	S
UT	R	R	R	S	I	R	S	S	I	R	S	I
UT	R	I	R	S	S	R	S	R	I	R	S	S
0141	R	S	R	I	S	S	S	R	R	S	S	S
07	R	S	R	I	S	R	S	R	R	I	S	S
040	R	S	R	S	S	S	S	R	R	R	I	I
087	R	S	R	I	R	R	S	R	R	R	S	R
029	R	S	R	I	S	R	I	R	I	R	S	I
08	R	S	S	R	R	R	R	S	R	R	S	S
029	R	I	S	R	S	R	I	R	R	R	R	I
08	R	S	R	S	S	S	R	R	R	R	S	I
060	R	S	R	S	S	I	I	R	R	R	R	S
0172	R	I	R	S	S	I	S	R	R	R	S	I
042	R	I	R	I	R	R	S	R	R	I	S	S
02	R	S	S	I	S	S	R	I	R	R	R	I
Rough	R	S	R	S	S	R	S	S	R	S	S	S
0148	R	S	R	R	S	I	S	R	R	R	I	I
042	R	S	R	R	S	I	S	R	R	R	I	R
Rough	R	S	R	S	S	R	S	R	R	R	S	S
01	R	S	R	I	S	I	S	S	R	R	I	I

Cu = Cefuroxime; G = Gentamicin; A = Ampicillin; Ci = Ceftriaxone; Ak = Amikacin; Ch = Cephalothin; Cf = Ciprofloxacin; Co = Co-trimoxazole; P = Penicillin; Va = Vancomycin; Of = Ofloxacin; T, Tetracycline; S = Susceptible; I = Intermediate; R = Resistant; UT = Untypeable.

Table III. Oligonucleotide primers used in multiplex PCR reaction.

Antibiotic		Number of resistant strain (n = 46)	Percent
β lactams antibiotic	Ampicillin	37	80.4
	Penicillin	41	89.13
Cephalosporins	Cephalothin (First generation)	28	60.86
	Cefuroxime (Second generation)	41	89.13
	Ceftriaxone (Third generation)	13	28.26
Fluroquinolones	Ciprofloxacin	3	6.52
	Ofloxacin	6	13.04
Aminoglycosides	Amikacin	6	13.04
	Gentamicin	6	13.14
Sulfonamides	Co-trimoxazole	34	73.9
Tetracyclines	Tetracycline	8	17.39
Glycopeptides	Vancomycin	34	74.1

Discussion

Foodborne diseases are caused by a number of pathogens. Of these, *E. coli* is considered one of the most important. It is responsible for intestinal and extraintestinal diseases in humans (Vincent *et al.* 2010, Scallan *et al.* 2011). Food plays an important role in the dissemination of *E. coli*, causing community-acquired urinary tract infections (Vincent *et al.* 2010, Ferens and Hovde 2011, Jana and Mondal 2013). In the present study, poultry meat samples collected from different retail shops were contaminated with different serogroups of *E. coli*. Out of 228 poultry meat samples, 62 (27.19%) were positive for *E. coli*. This finding is both consistent with previous reports from different parts of India (Farooq *et al.* 2009, Bonyadian *et al.* 2011, Sahoo *et al.* 2012, Jana and Mondal 2013) but higher than the finding of Zende and colleagues (Zende *et al.* 2013), who reported the isolation of *E. coli* in 16% poultry meat from a small sample size of meat from Mumbai, India. Our results indicate the contamination of poultry meat with *E. coli*. This may be due to a lack of proper sanitation during slaughtering in the local markets or because of contaminated water is used to wash carcasses.

In the present study, *E. coli* isolates belonged to 24 different serogroups, namely O29, O8, O40, O2, O60, O106, O42, O87, O1, O7, O30, O45, O59, O66, O105, O116, O136, O141, O147, O148, O166, O172; UT (untypable) and R (rough types) were also recovered. Most of the serogroups identified have also been reported in other similar studies

Table IV. Resistance pattern of *E. coli* serogroups isolated from poultry meat.

Resistance patterns	<i>E. coli</i> serotypes
CU-CO-VA	2
A-CU-CH	1
G-A-P-CH-VA	1
CU-CO	1
G-P-CU-CH-CO-VA	1
P-CU-CH	1
A-P-CU-VA	2
P-CI-CU-CO-VA	1
A-P-CI-CU-CH-VA	1
A-P-CI-CU-CH-CO-VA-T	1
A-P-CU-CO-VA-T	1
A-P-CU-VA-T	1
A-P-CI-CU-CO-VA-T	1
A-P-CH-CO-VA	1
A-P-CI-CH	1
A-P-CU-CH-CO-VA-T	2
G-AK-A-P-CI-CH-CO-T	1
G-A-P-CI-CU-CH-CO-VA	2
A-P-CU-CH-CO	3
G-A-P-CU-CO-VA	2
G-A-P-CO-VA	1
A-P-CU-CH-OF-VA	1
A-P-CU-CH-CO-VA	3
A-P-CU-CH-OF-CO-VA	1
A-P-CU-CH-CO	4
A-P-CO-VA	1
A-CU-CH-CO-VA-T	1
A-CU-CH-CO-VA	1
P-CU-CH-CF-VA	1
P-CU-CH-OF-CO-VA	1
A-P-CU-CF-CO-VA	1
A-P-CU-OF-CO-VA	1
P-CU-CH-CF-OF-VA	1
A-P-CU-CH	1
Total	46

Antibiotics tested for multiple resistance were: Cu = Cefuroxime; G = Gentamicin; A = Ampicillin; Ci = Ceftriaxone; Ak = Amikacin; Ch = Cephalothin; Cf = Ciprofloxacin; Co = Co-trimoxazole; P = Penicillin G; Va = Vancomycin; Of = Ofloxacin; T = Tetracycline.

(Guth *et al.* 1989, Blanco *et al.* 2004, Fratamico *et al.* 2010, Bandyopadhyay *et al.* 2011). In the present study however 24 serogroups were detected. They were widely distributed in the eastern part of India. On the contrary to this finding many studies from India reported the isolation of a few numbers of serogroups from poultry (Malik *et al.* 2002, Wani *et al.* 2004, Dutta *et al.* 2011). The *E. coli* of serogroups O2, O87, and O172 reported in this study, are associated with Shiga toxin producing *E. coli* (STEC), as documented by Fratamico and other authors (Fratamico *et al.* 2010, Meng *et al.* 2014), whereas the

serogroups O8, O29, O111, and O60 are associated to enteropathogenic *E. coli* (Guth *et al.* 1989, Blanco *et al.* 2004, Wani *et al.* 2004, Bandyopadhyay *et al.* 2011). Thus our findings indicate that meat handlers and consumers are at risk of infection from STEC or enteropathogenic *E. coli*.

In this study, molecular characterisation for the presence of *stx*₁ and *stx*₂ genes showed the presence of *stx*₂ gene in 13 (21%) isolates of *E. coli*. This finding is consistent with other findings from Farooq and colleagues (Farooq *et al.* 2009) and Grossmann and colleagues (Grossmann *et al.* 2005), who reported the *stx*₂ gene in 11% and 9% of *E. coli* isolated from pigeons, respectively. Various authors reported the occurrence of the *stx*₂ gene in O45, O18, O75, O64, O89, O91, O17, and O78 serogroups (Schmidt *et al.* 2000, Morabito *et al.* 2001, Farooq *et al.* 2009, Dutta *et al.* 2011). However, our findings demonstrate the presence of the *stx*₂ gene in O29, O8, O42, and O1 serogroups. This indicates a variable distribution of virulent genes among different serogroups of *E. coli* in different geographical area in India.

Antimicrobial resistance was observed at a range of 9-90%, which shows extreme variation in the susceptibility of poultry meat to *E. coli*. Our findings show maximum resistance against cefuroxime (a cephalosporin) followed by penicillin, ampicillin, and vancomycin. The high level of susceptibility against amikacin and ciprofloxacin that has been observed is consistent with the findings of other studies (Mishra *et al.* 2002, Carraminana *et al.* 2004, Jana and Mondal 2013). These findings might be the result of the indiscriminate use of these antibiotics in poultry or because of sub-therapeutic doses of these antimicrobial agents being administered for the prevention or control of infection. Other explanations include transmissible or plasmid mediated drug resistance and mutational changes in the genes that are crucial to the development

of resistance in *E. coli*. The coexistence of virulent and resistant genes in a species may result in the emergence of hybrid plasmids (both resistance and virulence) among *E. coli*. This, in turn, poses an increased public health risk (Szmolka and Nagy 2013). In the present study, all isolates of *E. coli* exhibited resistance against 2 or more antimicrobial agents, suggesting the presence of multi-drug resistant (MDR) *E. coli* (Joshi *et al.* 2012, Szmolka and Nagy 2013). The prevalence of such a large number of MDR *E. coli* indicates the widespread and extensive use of antibiotics in the area. Prevalence of such MDR *E. coli* has also been observed by Shakya and colleagues (2013) in human stool samples from India. The high rate of multiple antibiotic resistance against *E. coli* also indicates the injudicious use of several antimicrobial agents for preventive and therapeutic purposes. These all pose serious threats to animal and human health.

The present investigation highlights the prevalence of pathogenic *E. coli* (ETEC and EPEC) in poultry meat, which is of importance in the field of zoonosis. The occurrence of high levels of resistance against most of the antibiotics and prevalence of virulent genes call for further attention, and the maintenance of strict hygienic measures in retail meat shops. The present findings also provide information about the occurrence of STEC in poultry in eastern India.

Acknowledgments

The authors are thankful to the Honorable Vice Chancellor, Bihar Agricultural University, Sabour, for providing necessary funds and Principal, Bihar Veterinary College, Patna for providing infrastructure and facilities to carry out the investigation. We are also thankful to the Director CRI, Kasauli, HP, India, for serotyping the isolates.

References

- Bandyopadhyay S., Mahanti A., Samanta I., Dutta T.K., Ghosh M.K., Bera A.K., Bandyopadhyay S. & Bhattacharya D. 2011. Virulence repertoire of Shiga toxin-producing *Escherichia coli* (STEC) and enterotoxigenic *Escherichia coli* (ETEC) from diarrhoeic lambs of Arunachal Pradesh, India. *Trop Anim Health Prod*, **43** (3), 705-710.
- Blanco M., Blanco J.E., Mora A., Dahbi G., Alonso M.P., González E.A., Bernárdez M.I. & Blanco J. 2004. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-xi). *J Clin Microbiol*, **42** (2), 645-651.
- Boerlin P., McEwen S.A., Boerlin-Petzold F., Wilson J.B., Johnson R.P. & Gyles C.L. 1999. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol*, **37**, 497-503.
- Carraminana J.J., Rota C., Agustin I. & Herrera A. 2004. High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from a poultry slaughterhouse in Spain. *Vet Microbiol*, **104**, 133-139.
- Chattopadhyay U.K., Dutta S., Deb A. & Pal D. 2001. Verotoxin 6 producing *Escherichia coli* - an environment induced emerging zoonosis in and around Calcutta. *Int J Environ Health Res*, **11**, 107-112.
- Dutta T.K., Roychoudhury P., Bandyopadhyay S. Wani S.A. & Hussain I. 2011. Detection & characterization of Shiga toxin producing *Escherichia coli* (STEC) & enteropathogenic *Escherichia coli* (EPEC) in poultry birds with diarrhea. *Indian J Med Res*, **133**, 541-545.
- Edwards P.R. & Ewing W.H. 1972. Identification of enterobacteriaceae. 3rd Ed. Minneapolis, Burgess Publishing Company, 452-467.
- Farooq S., Hussain I., Mir M.A., Bhat M.A. & Wani S.A. 2009. Isolation of atypical Enteropathogenic *Escherichia coli* and shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India. *Lett Appl Microbiol*, **48**, 692-697.
- Ferens W.A. and Hovde C.J. 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog Dis*, **8**, 465-487.
- Fratamico P.M., Xianghe Y., Yanhong L., DebRoy C., Byrne B., Monaghan A., Fanning S. & Bolton D. 2010. *Escherichia coli* serogroup O2 and O28ac O-antigen gene cluster sequences and detection of pathogenic *E. coli* O2 and O28ac by PCR. *Canadian J Microbiol*, **56** (4) 308-316.
- Grossmann K., Weniger B., Baljer G., Brenig B. & Wieler L.H. 2005. Racing, ornamental and city pigeons carry Shiga toxin producing *Escherichia coli* (STEC) with different Shiga toxin subtypes, urging further analysis of their epidemiological role in the spread of STEC. *Berl Munch Tierarztl Wochenschr*, **118**, 456-463.
- Guth B.E., Silva R.M., Toledo M.R., Lima T.M. & Trabulsi L.R. 1989. Virulence factor and biochemical characteristic of serotypes of *E. coli* serogroup O29. *J Clin Microbiol*, **27**, 2161-2164.
- Hammerum A.M. & Heuer O.E. 2009. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. *Clin Infect Dis*, **48**, 916-921.
- Jana A. & Mondal A. 2013. Serotyping, pathogenicity and antibiogram of *Escherichia coli* isolated from raw poultry meat in West Bengal, India. *Vet Ital*, **49**, 361-365.
- Johnson T.J. & Nolan L.K. 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. *Microbiol Mol Biol Rev*, **73**, 750-774.
- Joshi S., Singh R. & Singh S.P. 2012. Antibiotic resistance profile of *E. coli* isolates from colibacillosis in and around Pantnagar, India. *Vet World*, **5** (7) 405-408.
- Khan A., Yamasaki S., Sato T., Ramamurthy T., Pal A., Datta S., Chowdhury N.R., Das S.C., Sikdar A., Tsukamoto T., Bhattacharya S.K., Takeda Y. & Nair G.B. 2002. Prevalence and genetic profiling of virulence determinants of non-O157 Shiga-toxin producing *Escherichia coli* isolated from cattle, beef and humans, Calcutta, India. *Emerg Infect Dis*, **8**, 54-62.
- Malik A.R., Rashid A., Qureshi A.M. & Wani S.A. 2002. Prevalence of 15 enterobacteria in migratory and non-migratory water birds in Srinagar. *J Res- SKUAST-J*, **1**, 75-78.
- Meng Q., Bai X., Zhao A., Lan R., Du H., Wang T., Shi C., Yuan X., Bai X., Ji S., Jin D., Yu Bo., Wang Y., Sun H., Liu K., Xu J. & Xiong Y. 2014. Characterization of Shiga toxin-producing *Escherichia coli* isolated from healthy pigs in China. *BMC Microbiol*, **14**, 5-18.
- Mishra A., Sharda R., Chhabra D. & Tanwani S.K. 2002. Antibiogram of *Escherichia coli* isolates from domestic poultry. *Indian Vet J*, **79** (8), 863-864.
- Morabito S., Dell'Omo G., Agrimi U., Schmidt H., Karch H., Cheasty T. & Caprioli A. 2001. Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. *Vet Microbiol*, **82**, 275-283.
- Overdevest I., Willemsen I., Ringsburger M., Eustace A., Xu Li., Hawkey P., Heck M., Savelkoul P., Vandenbroucke-Grauls C., van der Zwaluw K., Huijsdens X. & Kluytmans J. 2011. Extended-spectrum β lactamase gene of *E. coli* in chicken meat and humans, the Netherlands. *Emerg Infect Dis*, **17**, 1216-1222.
- Sahoo T.K., Sahoo L., Sarangi L.N., Panda S.K. & Panda H.K. 2012. Prevalence, isolation, characterization and antibiogram study of pathogenic *E. coli* from different poultry farms of Odisha. *J Adv Vet Res*, **2**, 169-172.
- Sambrook J., Fritsch E.F. & Maniatis T. 1989. Molecular cloning-A laboratory Manual. 2nd Ed. New York, Cold Spring Harbour Laboratory Press, Cold Spring Harbour.
- Scallan E., Griffin P.M., Angulo F.J., Tauxe R.V. & Hoekstra R.M. 2011. Foodborne illness acquired in the United States - unspecified agents. *Emerg Infect Dis*, **17** (1), 16-22.
- Schmidt H., Scheef J., Morabito S., Caprioli A., Wieler L.H. & Karch H. 2000. A new Shiga toxin 2 variant (stx2f) from *Escherichia coli* isolated from pigeons. *Appl Environ Microbiol*, **66**, 1205-1208.
- Shakya P., Barrett P., Diwan V., Marothi Y., Shah H., Chhari N., Tamhankar A. J., Pathak A. & Lundborg C.S. 2013. Antibiotic resistance among *Escherichia coli* isolates from stool samples of children aged 3 to 14 years from Ujjain, India. *BMC Infect Dis*, **13**, 477-482.

- Shekhar C. & Singh S.P. 2014. Antimicrobial resistance and molecular characterization of *Salmonella* serovars from man and animals. *Indian journal of animal sciences*, **84** (4), 368-372.
- Szmulka A. & Nagy B. 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. *Front Microbiol*, **4**, 258-278.
- Szmulka A., Anjum M.F., La Ragione R.M., Kaszanyitzky E.J. & Nagy B. 2012. Microarray based comparative genotyping of gentamicin resistant *Escherichia coli* strains from food animals and humans. *Vet Microbiol*, **156**, 110-118.
- Van de Bogaard A.E. & Stobberingh E.E. 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. *Int J Antimicrob Agents*, **14**, 327-335.
- Vincent C., Boerlin P., Daignault D., Dozois C M., Dutil L., Galanakis C., Reid-Smith R. J., Tellier P.P., Tellis P. A., Ziebell K. & Manges A.R. 2010. Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg Infect dis*, **16**, 88-95.
- Wani S.A., Samanta I., Bhat M.A. & Nishikawa Y. 2004. Investigation of Shiga toxin- producing *Escherichia coli* in avian species in India. *Lett Appl Microbiol*, **39**, 389-394.
- Wayne P.A. 2002. Performance standards of antimicrobial susceptibility. National Committee for Clinical Laboratory Standards (NCCLS). NCCLS approved standards, M 100-M 159.
- Zende R.J., Chavhan D.M., Suryawanshi P.R., Rai A.K. & Vaidya V.M. 2013. PCR detection and serotyping of enterotoxigenic and shigatoxigenic *Escherichia coli* isolates obtained from chicken meat in Mumbai, India. *Vet World*, **6** (10), 770-773.
- Zhao S., Blickenstaff K., Bodeis- Jones S., Gaines S.A., Tong E. & McDermott P.F. 2012. Comparison of the Prevalence and antimicrobial resistances of *Escherichia coli* isolates from different retail meats in the Unites States, 2002 to 2008. *Appl Environ Microbiol*, **78**, 1702-1707.