Detection of virulence gene and antimicrobial resistance of *E. coli* from poultry and poultry rearers

M. Rashid[1*], S. Gazal[2], I.A. Ganai[3]

[1]Division of Veterinary Public Health and Epidemiology, [2]Division of Veterinary Microbiology and Immunology, [3]Division of Animal Nutrition, F.V.Sc & AH, SKUAST-Jammu, India
*Corresponding Author's Email: rashidvph03@gmai.com

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Abstract

A study was conducted from July, 2021 to December, 2023 in Jammu, to investigate pathogenic '*Escherichia coli'* from poultry and poultry handlers, to determine the virulence genes profile and antimicrobial drug resistance pattern of the organism. A total of 150 samples comprising 50 poultry droppings, 50 cloacal swabs and 50 hand swabs from poultry handlers were collected and processed, yielding 102 *E. coli* isolates. The isolation rate of *E. coli* was 90% (45/50) from poultry droppings and 90% (45/50) from cloacal swabs, 24% (12/50) from hand swabs of poultry handlers. All 102 isolates of *E. coli* were screened for the virulence genes *stx1, stx2, eaeA* and *ehxA*, using multiplex polymerase chain reaction (mPCR). Of these, 11(10.78%) isolates carried one or more virulence genes. Notably all pathogenic isolates originated from poultry droppings and cloacal swabs whereas, none of the isolates recovered from poultry handlers possessed any of the target genes. Among the 11 pathogenic isolates from poultry droppings and cloacal swabs, 3 (27.27%) harbored *stx1,* 3 (27.27%) *eae*A gene, while 2 (18.18%) harbored both *stx1 a*nd *eaeA, 2*(18.18%) possessed s*tx2* and *eaeA* and one (9.09%) possessed both *eaeA* and *ehxA* gene. Antimicrobial susceptibility testing was performed against 10 commonly practiced antibiotics in field. The highest level of resistance was observed against Enrofloxacin (98.03%), followed by Cefotaxime (94.11%), while maximum sensitivity was noted to Amoxicillin/Clavulanic acid (95.05% and Gentamicin (80.39%). The finding indicates presence of pathogenic multidrug resistant *E. coli* in poultry, highlighting the potential public health risk associated with antimicrobial resistant bacteria in poultry production environment.

Key words: *Escherichia coli,* virulence genes, Poultry, handlers, Resistance.

Introduction

The *Escherichia coli* is commonly present as part of intestinal flora of most of birds, animal species and man (Ramos et al 2020). However, the bacterium has been implicated in the etiology of many diarrhoeal diseases and systemic infections in birds, animals and human beings (FAO/WHO 2018, Singh et al 2016, Navadeepa et al 2025). In poultry *E. coli* is responsible for causing many infections such as swollen head syndrome, omphalitis (yolk sac infection), and colibacillosis (Azam et al 2019). The *E. coli* possesses several pathogenic determinants such as shiga like toxins encoded by genes *stx1* and *stx2* and these toxins are also referred as verotoxins; intimin encoded by *eaeA* gene and entrohemolysins encoded by *ehxA* gene (Paton and Paton 1998). Shiga like toxins are responsible for causing serious disease conditions especially in immuno-compromised humans and children (Jourdan et al 2012). Intimin is a virulence – associated factor that helps in the attachment of the bacterium to epithelial cells of intestines, there by producing attaching and effacing lesions in the intestinal mucosa (Naidoo and Zishiri, 2025). Intimin encode by *eaeA* is a chromosomal gene which resides in the pathogenicity island called as – locus for enterocyte effacement. Another virulence determinant enterohemolysin encode by *ehxA* (Zishiri et al 2016) is found in some strains of organism alone or in combination with other genes. This pathogenic *E. coli* has been found associated with number of disease conditions – as endocarditis, meningitis, septicemia, urinary tract infections and diarrhea in human beings (Pakbin, et al 2021).

The detection of organism in foods of animal origin indicates contamination and may lead to the food spoilage in addition to causing various food borne outbreaks especially when such bacteria acquire drug resistance determinants by different mechanisms (FAO/WHO, 2018, Rehman and Ahmed, 2022). The transmission of pathogenic strains of *E. coli* to man could occur not only through poultry meat and meat products but also from their environment due to unhygienic conditions prevailing at the processing sites (Rashid et al 2013).

Faecal contamination of poultry farm environment favours re-infection of birds and therefore, persistence of the pathogens in the farm. Hence poultry production units represent a major reservoir for the *E. coli* and human infections can occur via faecal contamination of food products (Azam, 2019, Rehman and Ahmed, 2022). Therefore, to reduce the risk of human infections, it is critical to prevent bacterial population in live birds at the primary production site and finally elimination from the food chain. In comparison to the other developed countries, poultry farming in the union territory of Jammu and Kashmir is a house hold practice in an unorganized farming. To generate the data on the occurrence of pathogenic, multidrug resistant *E. coli,* from poultry and poultry rearers will play a significant role in understanding the epidemiology of organism and thereby in controlling the contamination of poultry meat. Such studies are poorly conducted in this Union Territory and antibiotic resistance in bacteria is a global threat. Considering the public health importance of pathogenic *E. coli,* this study was taken with the objective to explore the occurrence of organism from poultry and poultry associated environment of the study area.

Materials and Methods

One hundred and fifty samples (50 poultry droppings from environment, 50 cloacal swabs and 50 hand swabs of poultry rearers/ handlers) from different locations of Ranbir Singh Pura Block were collected for processing. During the preliminary survey the private unorganized poultry farms of Ranbir Singh Pura block of Jammu district were selected. The consent of poultry rearers/handlers was sought to participate voluntarily and it was ensured from us that their particulars will not be disclosed. But wherever, needed scientific measures will be implemented to overcome the problem in the interest of public. The samples were randomly collected in a cross-sectional study during July, 2021 to June, 2023. The samples were aseptically collected and transported to the laboratory maintaining the refrigeration conditions with ice packs and processed in the division of veterinary public health and epidemiology.

Isolation and Identification of *E. coli*

The swab sample or a loop of droppings was enriched in 90 ml sterile MacConkey's Lactose Broth (Himedia Pvt. Ltd. Mumbai India) and incubated at 37 °C for 12-24 hours. A loopful of inoculum was streaked onto MacConkey's Lactose Agar and incubated for 24-48 hour at 37 °C. Pink colonies were considered as lactose fermenting organisms. The suspected *E. coli* colonies showing the pink colour were selected and further inoculated on the Eosin Methylene Blue agar (EMB) (HiMedia Pvt. Ltd. India). Colonies exhibiting the characteristic greenish gold metallic sheen were further cultured on nutrient agar slants and further confirmed as per the standard microbiological techniques with slight modifications.

Extraction of Bacterial DNA

Extracted the deoxyribonucleic acid (DNA) of bacteria by snap chill technique. By suspending a loop of bacterial culture in 500µl sterile distilled water in a 2 ml microcentrifuge tube. Boiled the tube for 10 minutes in a water bath followed by chilling on ice and centrifugation at 10000 g for 5 minutes. 2.0 µl of supernatant was used as template DNA for mPCR.

Detection of virulence genes of *E. coli* by Multiplex Polymerase Chain Reaction

The presence of the virulence genes viz *stx1, stx2*. *eaeA, and ehxA,* were detected by multiplex PCR as described previously (Paton and Paton 1998) with slight modifications. The multiplex polymerase chain reaction was constituted in a volume of 25µl by adding 12.5 µl of (master mixture) IX Green GoTag Flexi buffer (Promega Pvt. Ltd.), 2mM $MgCl_2$, 0.2 mM of each 2′-deoxynucloside 5′triphosphate (dNTPs), 0.5 mM of each forward and reverse primer, Tag polymerase 1 unit (Promega) and 2.0 µl DNA template. The samples in the beginning subjected to denaturation at 95^0C for 2 minutes, followed by 15 cycles, each cycle consisting of denaturation at 95^0C for 1 minute, annealing at 65°C for 1 minute and elongation at 72°C for 1.5 minutes. A second phase of twenty cycles was performed with each cycle consisting of denaturation of one minute at 95^0C, annealing at 60^0C for two minutes, extension for two minutes at 72^0C, final extension was done at 72°C for 5 minutes. The PCR product was analyzed by agarose gel electrophoresis for amplicon sizes of 180 bp, 255 bp, 384 bp and 534 bp for *stx1, stx2, eaeA and ehxA* genes respectively by using 1.5% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under ultraviolet light in a gel documentation system (Bio-rad).

Antibiotic sensitivity assay

All the 102 *Escherichia coli* isolates obtained in the present research work were tested against the ten commonly used antibiotics in poultry practice in the private farming practices as per the information collected during collection of samples. The sensitivity assay was carried out by using the Muller Hinton Agar plates by the disc diffusion method as described previously (Bauer et al 1966). The susceptibility/resistance patterns of bacteria against the antibiotics viz. Amoxicillin/Clavulanic acid, Ampicillin, Cefotaxime, Ciprofloxacin, Enrofloxacin, Polymixin-B, Tetracycline, Gentamycin, Norfloxacin, and Nalidixic acid was determined. The bacterial strains were designated as sensitive, intermediate or resistant to an antibiotic, based on the diameter of zone of inhibition interpreted as per the guidelines of disc manufacturers (HiMedia Pvt.Ltd. India).

Results

The isolates exhibiting characteristic pink colonies on MacConkey's Lactose agar (MLA) and greenish golden metallic sheen on Eosin Methylene Blue Agar were recognized as *E. coli* and further characterised based on standard microbiological tests. The 102 isolates obtained on processing 150 samples were confirmed by the standard microbiological procedures.

Out of 102 isolates, 90 were from poultry droppings and poultry cloacal swabs (45 each), 12 were from hand swab samples collected from poultry rearers/ handlers.

Detection of pathogenic genes by Multiplex Polymerase Chain Reaction

All the 102 isolates obtained were screened for the presence of genes viz. *stx1, stx2, eaeA* and *ehxA* by using, 180 bp, 255 bp, 384 bp and 534 bp primers respectively. Out of them 11(10.78%), strains were found to carry virulence gene. From the 90 strains obtained from poultry droppings and cloacal swabs, 11 were positive for one or more genes alone or in combination. The 3 isolates from poultry droppings harboured *stx1* gene and 3 isolates harboured *eaeA,* one isolate was having both *eaeA and ehxA* gene,2 were having both *stx1* and *eaeA* gene, while 2 isolates were possessing *stx2 and eaeA* gene (figure-1).

Out of 12 isolates from hands swabs of poultry rearers none was possessing *stx1, stx2, eaeA* or *ehxA* gene. The detail of all these four genes viz. *stx1, stx2, eaeA*, and *ehxA* has been presented in table-2. All the strains of *E. coli* are not pathogenic, only the strains that belong to one of the six pathotypes or possessing the pathogenic characters are pathogenic ones. The investigator collected the hand swabs from apparently healthy poultry handlers, who might have taken precautions while handling diseases birds or their droppings. The *E. coli* isolated from their hand swabs may be contamination of water utilized in day to day practices or may be environment contaminants.

Antibiotic sensitivity assay

The antibiotic susceptibility pattern of *Escherichia coli* obtained in the study revealed maximum resistance to Enrofloxacin (98.03% -100 isolates resistant), followed by Cefotaxime (94.11% - 96 isolates resistant), Nalidixic acid (58.82% - 60 isolates resistant), Norfloxacin (55.88% - 57 isolates resistant), Tetracycline 50.98% - 52 isolates resistant), Ciprofloxacin (33.33% - 34 isolates resistant), and Ampicillin 58.82% - 60 isolates resistant). Maximum sensitivity of *E. coli* isolates from poultry was noted against Amoxicillin/Clavulanic acid 95.09% - 97 isolates sensitive), Gentamycin 80.39% - 82 isolates sensitive) Ciprofloxacin 60.78% - 62 isolates sensitive), Ampicillin 58.82% - 60 isolates sensitive, Polymixin –B 56.86% - 58 isolates sensitive). The detail of each of the antibiotic, sensitivity/resistance pattern is explained in the figure-2.

Table-1: List of Primers (5'-3') used in the multiplex PCR

Primer	***Sequence***	***Amplicon size***
stx1-F	*ATAAATCGCCATTCGTTGACTAC*	*180 bp*
stx1-R	*AGAACGCCCACTGAGATCATC*	
stx2-F	*GGCACTGTCTGAAACTGCTCC*	*255 bp*
stx2-R	*TCGCCAGTTATCTGACATTCTG*	
eaeA-F	*GACCCGGCACAAGCATAAGC*	*384 bp*
eaeA-R	*CCACCTGCAGCAACAAGAGG*	
ehxA-F	*GCATCATCAAGCGTACGTTCC*	*534 bp*
ehxA-R	*AATGAGCCAAGCTGGTTAAGCT*	

Table-2: Profile of virulence genes of *Escherichia coli*

	Sample	**No. of Isolates**	***E. coli* virulence genes screened**						
			stx1	*stx2*	*eaeA*	*ehxA*	*Stx1+eaeA*	*Stx2+eaeA*	*eaeA+ehxA*
Poultry	Poultry Droppings & cloacal swabs	90 (45 each)	3	-	3	-	2	2	1
Poultry Rearers	Hand swabs	12	-	-	-	-	-	-	-
Total		**102**	**3**	**-**	**3**	**-**	**2**	**2**	**1**

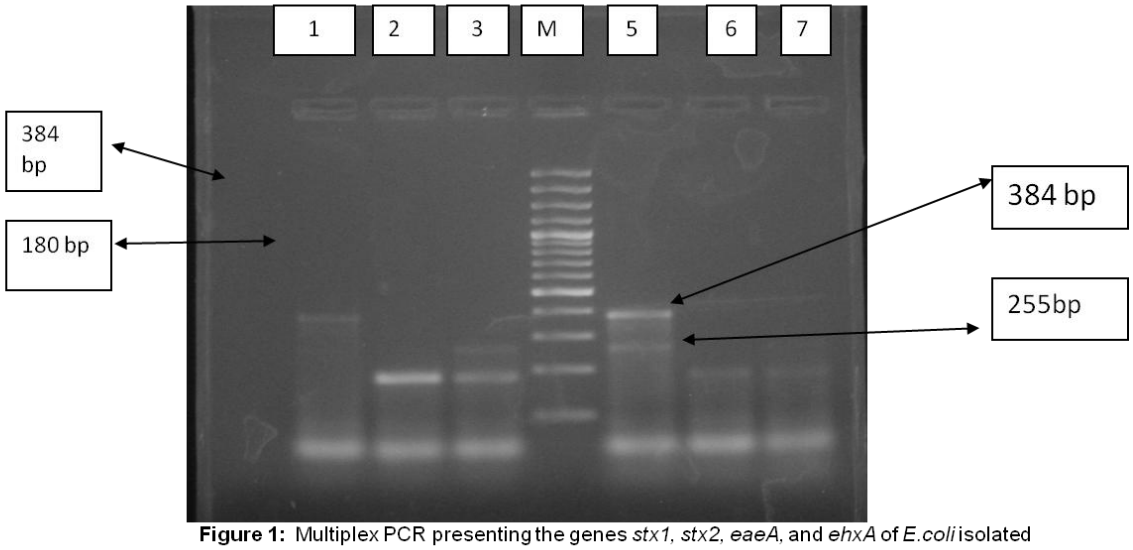

Figure 1: Multiplex PCR presenting the genes *stx1*, *stx2*, *eaeA*, and *ehxA* of *E.coli* isolated from poultry and poultry Rearers. Lane M is 100 bp marker ladder, Lane L3 shows positive control, revealing genes *stx1 and stx2* having the amplicon size of 180 bp, 255 bp, respectively. Lane L1 shows presence of *eaeA* (384 bp) L2 shows stx1 (180 bp) gene and L6 *stx1* (180 bp) gene and L5 shows presence of stx2 (255 bp) and *eaeA* (384 bp).

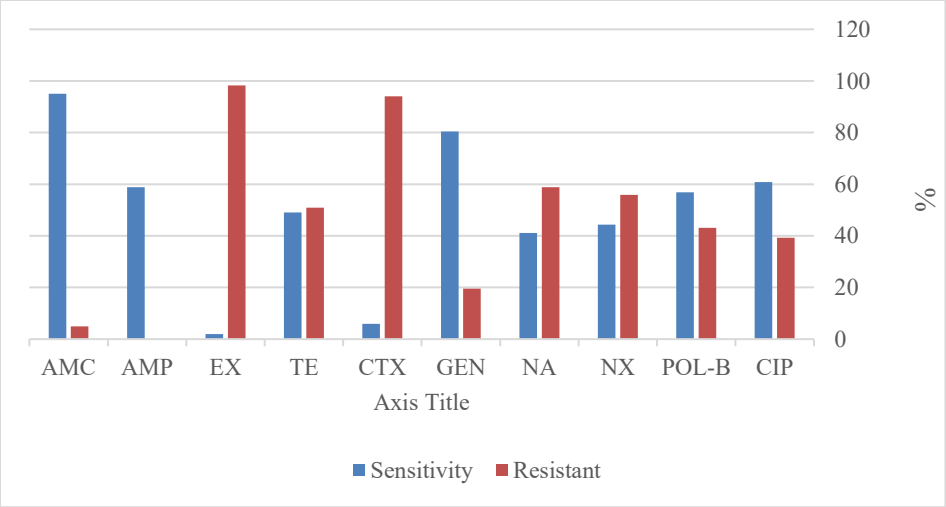

Figure -2: Antimicrobial Sensitivity Pattern of *E. coli* isolates from Poultry and their Rearers/Handlers

AMC- Amoxicillin/Clavulanic acid, 30µ, AMP- Ampicillin, 10µg
EX- Enrofloxacin 10µg, TE- Tetracycline, 30µg
CTX- Cefotaxime , 30µg, GEN- Gentamicin ,10µg
NA- Nalidixic acid, 30µg NX- Norfloxacin, 10µg
Pol-B- Polymyxin –B 300 units/disc CIP- Ciprofloxaxacin 5µg

Discussion

Escherichia coli is the food borne pathogen, usually transmitted through the contaminated foods or water among the animals, birds and human beings (Rashid. et al 2013). In the present study, a total of 150 samples from poultry and poultry rearers/handlers were collected. The samples included poultry droppings, poultry cloacal swabs and poultry rearers/ handlers hand swabs. Out of 150 (50 poultry droppings, 50 poultry cloacal swabs and 50 hand swabs) samples, we got 90 strains from poultry droppings and poultry cloacal swabs (45 from each), 12 strains from hand swabs. All the 102 isolates were screened for presence of multiple virulence genes. 11 of 102 (10.78%) of the isolates revealed one or more gene, from *stx1, stx2, eaeA,* and *ehxA* genes. The presence of virulence genes in this study was slightly lower in comparison to a study conducted at Andhra Pradesh (India), where a prevalence rate of 12% was reported (Sekhar et al2017). All the pathogenic strains obtained were from droppings and cloacal swabs whereas, none of isolate from hands swabs was found possessing any pathogenic gene. It indicates environmental contamination of hands of workers working in farms. Out of 11 pathogenic isolates from poultry droppings and cloacal swabs 3(27.27%) were having *stx1* gene and 3(27.27%) were possessing *eaeA* gene, two (18.18%) isolates were carrying *stx1* and *eaeA* gene in association and 2 (18.18%), isolates were also harbouring *stx2* and *eaeA* gene, one isolate (9.09%) harbored *eaeA and ehxA* gene. Thus, the overall presence of alone virulent genes, *stx1, stx2, eaeA* and *ehxA* was 3.33% (3/90), 0.0% (0/90), 3,33% (3/90) and 0.0% (0/90), respectively.

All the *E. coli* bacterial isolates obtained in the present study were screened against the ten commonly used antibiotics in poultry medicine. The isolates demonstrated maximum sensitivity to Amoxicillin/Clavulanic acid 95.09%), Gentamycin 80.39%), Ciprofloxacin 60.78 %), and Polymixin –B 56.86%). These results are in close association with our earlier study (Rashid et al2013), where the STEC were showing 60% sensitivity with Ciprofloxacin.

Conclusion

A total of 102 isolates were obtained from poultry and poultry environment, of which 10.78% carried one or more virulent genes, indicating pathogenic strains in private poultry farms of R.S. Pura block. None of the poultry handlers hands swab possessed the pathogenic strain. The isolates showed high resistance to enrofloxacin and cefotaxime, while showed good susceptibility to amoxicillin/clavulanic acid and gentamicin. These findings highlight poultry as a potential reservoir of pathogenic and antimicrobial resistant E. coli, emphasizing the need for continuous surveillance and judicial use of antibiotics in poultry production.

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Conflict of Interest

None of the authors have any potential conflict of interest to declare.

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Authors' contribution

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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