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Molecular detection of drug resistance and virulence related genes in avian pathogenic *Escherichia coli* (APEC) isolated from poultry

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Abstract
This study examined the occurrence of drug resistance and virulence-associated genes in 25 isolates of avian pathogenic *Escherichia coli* (APEC). A total of five genes linked to antimicrobial resistance *strA*, *strB*, *tetA*, *tetB*, and *blaTEM* were selected based on the phenotypic resistance observed to the corresponding antibiotics. A relatively high prevalence rate (92%) was found for the *blaTEM* gene. Moderate occurrence rates were recorded for *strB* (64%), *tetB* (56%), *tetA* (44%), and *strA* (40%).
In addition, these *E. coli* isolates were screened for virulence genes. The *iucD* gene exhibited the highest prevalence (68%), followed by *kpsMT II* (60%), *iss* (48%), *tsh* (44%), *vat* (40%), *papC* (32%), *irp2* (32%), and *astA* (28%). The *cva* gene was not detected in any of the 25 isolates.

Keywords: APEC, EXPEC, *E. coli*, PCR, poultry, pathogenic, drug resistance, avian pathogenic

Introduction
Escherichia coli (*E. coli*) can be classified into three categories from a clinical perspective: (a) commensal *E. coli*, (b) intestinal pathogenic *E. coli*, and (c) extraintestinal pathogenic *E. coli* (ExPEC). Four major pathotypes of intestinal *E. coli* are recognized: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC). Strains that cause infections outside the intestinal tract are referred to as ExPEC (Russo and Johnson, 2000) [11]. ExPEC is known to cause septicemia and systemic infections in birds (Kariyawasam *et al*., 2006; Russo and Johnson, 2003) [6, 12].
E. coli is classified into six phylogenetic groups: A, B1, B2, C, D, and E. The majority of ExPEC strains primarily belong to group B2, and to a lesser extent, group D (Smith *et al*., 2007) [13]. From phylogenetic and epidemiological perspectives, ExPEC strains can differ significantly from intestinal pathogenic and commensal strains. Most ExPEC strains possess multiple virulence factors that aid in various stages of infection, including adhesion, invasion of host tissues, evasion of host immune responses, and toxin production that disrupts normal cellular functions.
The inappropriate and excessive use of antibiotics is a key factor driving the emergence and spread of antimicrobial resistance. Selective pressure from antimicrobials contributes to the dissemination of multidrug resistance in avian *E. coli*. The increasing prevalence of drug-resistant traits in avian pathogenic *E. coli* (APEC) may be attributed to both genetic and non-genetic mechanisms. This study contributes to a better understanding of the virulence factors and multidrug resistance challenges posed by *E. coli*.

Materials and Methods
Pathogenic *Escherichia coli* isolates obtained from various samples and preserved as pure cultures were subjected to DNA extraction. The extracted DNA was then used for Polymerase Chain Reaction (PCR) assays employing specific primers as described by Bali *et al*. (2010) [2], Randall *et al*. (2004) [10] and Lanz *et al*. (2003) [8], for the detection of antimicrobial resistance genes including *strA*, *strB*, *tetA*, *tetB*, and *blaTEM* (Table 1).

To detect extraintestinal virulence-associated genes namely *astA*, *papC*, *iss*, *irp2*, *iucD*, *tsh*, *vat*, and *cvaA/B/cvi/cvaC* PCR was performed following the protocol by Ewers *et al*. (2005) [3]. Additionally, a uniplex PCR targeting the *kpsMTII* gene was carried out using the method described by Johnson and Stell (2000) [11], (Table 1).

The PCR products were electrophoresed on a 1.5% agarose gel. The amplified products appeared as single distinct bands of expected sizes under UV illumination and were documented using a gel documentation system.

Results and Discussion

A total of 25 multidrug-resistant *Escherichia coli* isolates were subjected to PCR amplification for the detection of *blaTEM*, *strA*, *strB*, *tetA*, and *tetB* genes. Out of the 25 isolates, 23 (92%) yielded a 403 bp product corresponding to the *blaTEM* gene, which encodes a beta-lactamase enzyme responsible for resistance to beta-lactam antibiotics (Table 2). These findings are consistent with previous reports by Wang *et al*. (2013) [14] and Ammar *et al*. (2015) [1], who detected the *blaTEM* gene in 65.9% and 100% of *E. coli* isolates, respectively, both reporting resistance to beta-lactam antibiotics. The present study confirms that *E. coli* isolates from broilers affected by colibacillosis frequently harbor *blaTEM*-mediated resistance.

Tetracycline resistance has become increasingly prevalent, largely due to its extensive use in poultry production. In this study, the isolates were analyzed by PCR for the presence of tetracycline resistance genes, specifically *tetA* and *tetB*. Out of 25 isolates, 11 (44%) produced a 577 bp amplicon for the *tetA* gene, while 14 (56%) yielded a 634 bp product for the *tetB* gene (Table 2). These findings align with the results of Momtaz *et al*. (2012) [9], who reported a 52.63% prevalence of both *tetA* and *tetB* in *E. coli* isolates from chickens. Similarly, Wilkerson *et al*. (2004) [15] found that 60% of tetracycline-resistant strains carried the *tetB* gene, identifying it as the most prevalent *tet* gene.

For streptomycin resistance, PCR detection of *strA* and *strB* genes yielded amplicons of 546 bp and 509 bp, respectively. Out of the 25 isolates, 10 (40%) were positive for *strA*, and 16 (64%) for *strB* (Table 2). These results fall within the range reported by Kim *et al*. (2007) [7], who also documented the presence of *strA* and *strB* genes among avian *E. coli* isolates.

Prevalence of virulent genes in APEC

Nine virulence genes associated with extraintestinal infections were screened by PCR in 25 *Escherichia coli* isolates. The detection rates of these genes are summarized as follows: the *iucD* gene showed the highest prevalence, detected in 17 isolates (68%), followed by *kpsMTII* in 15 isolates (60%), *iss* in 12 (48%), *tsh* in 11 (44%), *vat* in 10 (40%), *papC* in 8 (32%), *irp2* in 8 (32%), and *astA* in 7 (28%). The *cva* genes were not detected in any of the isolates (Table 3).

The findings of the present study partially align with those reported by Janben *et al*. (2001) [4], who observed a 92.7% prevalence of the *fimC* gene, followed by *iucD* (88.7%), *tsh* (85.3%), and other commonly detected genes such as *fyuA* (66.0%) and *irp2* (68.0%). Additionally, they reported the presence of *papC* in 30.0% and *astA* in 17.3% of field strains. Notably, a significant proportion of their isolates (57.3%) harbored a combination of *iucD*, *tsh*, *fimC*, and *irp2* genes, contributing to the overall virulence of the strains.

Table 1: Primer sequences used in PCR

Primer name	Oligonucleotide sequence	Target gene & Amplicon size (bp)	Reference
astA f	TGC CAT CAA CAC AGT ATA TCC	*astA* 116	Ewers *et al*., 2005 [3]
astA r	TCA GGT CGC GAG TGA CGG C		
papC f	TGA TAT CAC GCA GTC AGT AGC	*papC* 501	
papC r	CCG GCC ATA TTC ACA TAA		
iss f	ATC ACA TAG GAT TCT GCC G	*Iss* 309	
iss r	CAG CGG AGT ATA GAT GCC A		
irp 2 f	AAG GAT TCG CTG TTA CCG GAC	*irp2* 413	
irp 2 r	AAC TCC TGA TAC AGG TGG C		
iucD f	ACA AAA AGT TCT ATC GCT TCC	*iucD* 714	
iucD r	CCT GAT CCA GAT GAT GCT C		
tsh f	ACT ATT CTC TGC AGG AAG TC	*tsh* 824	
tsh r	CTT CCG ATG TTC TGA ACG T		
vat f	TCC TGG GAC ATA ATG GTC AG	*Vat* 981	
vat r	GTG TCA GAA CGG AAT TGT		
cva A/B	TGG TAG AAT GTG CCA GAG CAA G	*cva A/B cvi cvaC* 1181	
cvi cvaC	GAG CTG TTT GTA GCG AAG CC		
KpsMT II f	GCG CAT TTG CTG ATA CTG TTG	*KpsMT II* 272	Johnson and Stell., 2000 [11]
KpsMT II r	CAT CCA GAC GAT AAG CAT GAG CA		
bla*TEM* –F	TTT-CGT-GTC-GCC-CTT-ATT-CC	bla*TEM* 403	Bali *et al*., 2010 [2]
bla*TEM* –R	ATC-GTT-GTC-AGA-AGT-AAG-TTG-G		
tet A F	GGTTCACTCGAACGACGTCA	*tet A* 577	Randall *et al*., 2004 [10]
tet A R	CTGTCCGACAAGTTGCATGA		
tet B F	CCTCAGCTTCTCAACGCGTG	*tet B* 634	
tet B R	GCACCTTGCTGATGACTCTT		
str A F	**C**CTGGTGATAACGGCAATTC	*str A* 546	Lanz, *et al.*, 2003 [8]
str A R	CCAATCGCAGATAGAAGGC		
str B F	ATCGTCAAGGGATTGAAACC	*str B* 509	

Table 2: Detection drug resistance related genes of *E. coli*

Name of the gene	Total no of *E. coli* isolates	Total no of *E. coli* positive	Percentage of *E. coli* positive for drug resistance related genes
bla$_{TEM}$ *gene*	25	23	92.00%
*strB*gene	25	16	64.00%
*tet B*gene	25	14	56.00%
tetA gene	25	11	44.00%
strA gene	25	10	40.00%

Table 3: Detection of virulence related genes of *E. coli*

Name of virulence related gene	Total no of *E. coli* isolates	Total no of *E. coli* positive	Percentage of *E. coli* positive for virulence related genes
iucD gene	25	17	68.00%
KpsMT II gene	25	15	60.00%
iss gene	25	12	48.00%
tsh gene	25	11	44.00%
vat gene	25	10	40.00%
*pap*C gene	25	8	32.00%
irp 2 gene	25	8	32.00%
*ast*A gene	25	7	28.00%
cva gene	25	0	0.00%

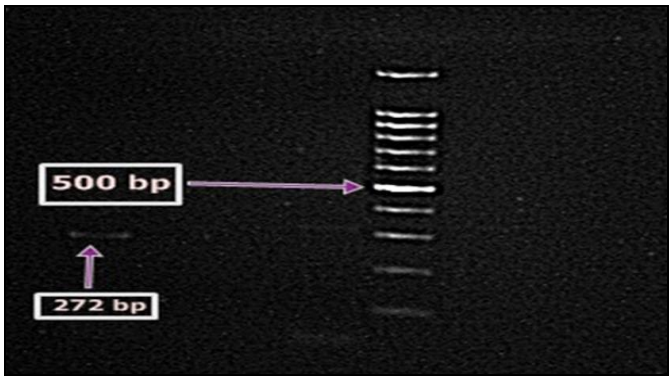

Plate 1: Amplified PCR product of *kpsMT II* gene 272 bp 100 bp DNA

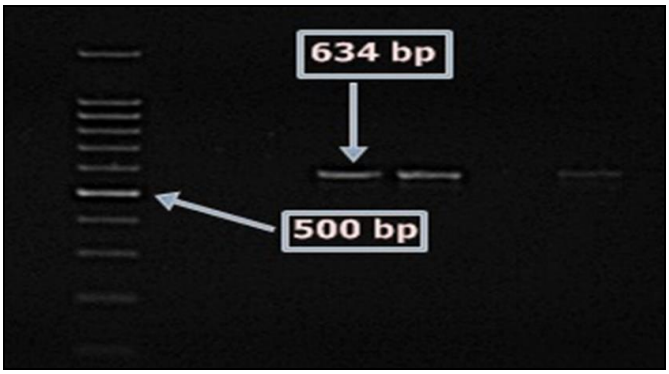

Plate 2: Amplified PCR product of *tetB* 634 bp 100 bp DNA ladder

Conclusions

The screening for drug resistance gene by PCR in *E. coli* isolates revealed high prevalence rate of bla$_{TEM}$ gene 92.00% followed by 40%, 64%, 44%, 56% of strA, *strB*, *tetA* and *tetB*, respectively. Whereas the screening for the virulence related genes of *E. coli* isolates revealed the highest percentage of *iucD* (68%), followed by kpsMT II gene (60%), iss gene (48%), *tsh* gene (44%), vat (40%), papC (32%), irp2 (32%), astA (28%). The cva gene was not found in any of *E. coli* the isolates

Conflict of Interest

Not available

Financial Support

Not available

Reference

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