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Original Research

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Detection of Antibiotic and Disinfectant Resistant Genes in *E. coli* Isolated from Broilers Chickens

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Abstract

Avian colibacillosis is one of the most serious diseases that affect poultry and causes substantial morbidity and mortality rates as well as high economic losses. *E. coli* is capable of acquiring resistance genes via gene transfer. The development of extended spectrum lactamases, or broad-spectrum lactamases, in *E. coli* is the most serious resistance mechanism. The goal of the current study was to detect the resistance associated genes of multi drug resistant *E. coli* isolated from broiler chicken by using PCR technique. In the current study PCR applied on 10 multidrug resistant *E. coli* isolates for detecting β-lactamases resistance genes (bla_{TEM}, bla_{SHV}), integron resistance gene (*Int1*, *Int3*), PCR was also used to detect disinfectant resistance genes as Quaternary ammonium compounds resistance genes (*QacCD*, *QacA/B* and *QacED1*). PCR results for antibiotic resistance associated gene showed that (10/10) of tested isolates had bla_{TEM} and *Int1*(10/10), bla_{SHV} (6/10) and *Int3* (2/10) also PCR results for disinfectant resistance associated genes showed that (8/10) of *E. coli* isolates had *QacED1*, *QacCD* (2/10), and *QacA/B* (2/10). The (10) broilers flocks investigated in the study were infected with multi drug resistant, strains of *E. coli*, that haboured β-lactamases, integron resistance associated gene and quaternary ammonium compounds resistance genes.

KEYWORDS
Antibiotic resistance, Broiler, β-lactamases, *Escherichia coli*, Integron

INTRODUCTION

Avian colibacillosis that caused by avian pathogenic *E. coli* (APEC) is one of the major problems in poultry houses. It's demonstrated in many forms as coligranuloma, enteritis, colisepticemia, chronic respiratory disease (CRD, air sacculitis), swollen-head syndrome, cellulitis, salpingitis, omphalitis/yolk sac infection, panophthalmitis, osteomyelitis, pericarditis, synovitis and peritonitis which frequently coexist with various types of bacteria, viruses, protozoa, and fungus (Yue *et al*., 2018).

Using of antibiotics is the first line used for prevention and control of *E. coli* infection and by excessive and improper use of antibiotics, the antibiotic resistance patterns are displayed by avian pathogenic *E. coli* in a form of resistance to aminoglycosides, sulfonamides, chloramphenicol, tetracyclines, penicillin and fluoroquinolones (Rahman *et al*., 2020). The most effective antibiotic should be chosen based on antibiotic sensitivity profile that are determined by laboratory testing.

One of the main issues facing health authorities worldwide is the dealing with antimicrobial resistance (AMR). Since AMR bacteria can spread throughout ecosystem, One Health strategy is necessary. However, it is now evident that AMR is a problem that affects people, animals, and the environment. The widespread use of antimicrobials and their improper application in both human and animal are directly related to the emergence and spread of AMR (Koutsoumanis *et al*., 2021).

The antimicrobial resistance bacteria play a role in transmission of resistance genes among commensal microorganisms (Álvarez *et al*., 2013). The final line of defense against *E. coli* infection is accomplished by using disinfectants as Quaternary ammonium compounds (QACs) that often are used in environments where antibiotics used that raising concerns about a link between QAC and antibiotic resistance (Hegstad *et al*., 2010).

Quaternary ammonium compounds (QACs) are cationic surface active detergents often utilized for the prevention of microorganisms in clinical and industrial settings to disinfect hard surfaces (Ioannou *et al*., 2007). QAC resistance genes were often present in *E. coli* isolates. Antimicrobial resistance phenotypes were strongly correlated with the qac genes (Zhang *et al*., 2016). The main means by which resistance genes are disseminated among the bacterial population are plasmids (Radwan *et al*., 2016).

There are many different *E. coli* strains that are resistant to different antimicrobials, and PCR can be used to detect antimicrobial resistance genes. Therefore, the goal of the current study was to detect resistance associated genes in multidrug resistant *E. coli* isolated from broiler chickens using PCR technique.

MATERIALS AND METHODS

Selected isolates for PCR

Ten *E. coli* isolates which were isolated from diseased broiler were selected for investigating the presence of bla_{TEM}, bla_{SHV},

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integron1, integron3, *QacCD*, *QacA/B* and *QacED1* genes by Polymerase chain reaction (PCR) . Isolates were selected on their proven Multidrug resistance profile determined by disc susceptibility testing.

Extraction of DNA

DNA was extracted from culture broth using and following the manufacturer instructions of QIAamp DNA Mini Kit (Qiagen, Germany, GmbH Catalogue No. 51304).

Polymerase chain reaction (PCR)

PCR reaction volume was as follows; 5 µL of the DNA template, 1 µL of each primer (20 pmol), 5.5 µL of diethyl pyrocarbonate water, and 12.5 µL of EmeraldAmp MAX PCR Master Mix (Takara) Code No. RR310A were added to the final 25 µL volume for the polymerase chain reaction.

Nine pairs of oligonucleotide primers specific for the following antimicrobial drug and disinfectant resistance genes: bla_{TEM}, bla_{SHV}, integron1, integron3, *QacCD*, *QacA/B* and *QacED1* were used for PCR, the primers were supplied from metabion (Germany) or Biobasic (Canada), primer sequences are shown in Table 1.

For each targeted PCR product, Temperature and time conditions of primers during PCR shown in Table 2.

Polymerase chain reaction products were separated by electrophoresis on a agarose gel 1.5 gram in 100 ml TBE buffer at room temperature for polymerization (Sambrook and Fritscgh, 1989). Each well was loaded with 15 µL of the PCR product. A GelPilot 100 bp (Qiagen) ladder was used to determine the fragment sizes by using Gene ruler 100 bp DNA ladder (cat. no. SM0243). The gel was photographed using a gel documentation system, data was analyzed through computer software.

RESULTS

PCR results for antibiotic resistance –associated gene showed that the tested isolates haboured β-lactamases resistance genes. The tested isolates had bla_{TEM} , *Int1*(10/10), bla_{SHV}(6/10) and *Int3* (2/10). PCR results for disinfectant resistance associated genes detected Quaternary ammonium compounds resistance genes in the tested isolate as the follow; (8/10) of *E. coli* isolates had *QacED1*, *QacCD* (2/10), and *QacA/B* (2/10), as shown in Tables 3, and Figs. 1-7.

Table 1. Oligonucleotide primers sequences.

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
Int1	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	280 bp	Kashif *et al*. (2013)
Int3	AGTGGGTGGCGAATGAGTG TGTTCTTGTATCGGCAGGTG	484 bp	
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al*. (2003)
bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp	
QacED1	TAA GCC CTA CACAAA TTG GGA GAT AT GCC TCC GCA GCG ACT TCCACG	362 bp	Chuanchuen *et al*. (2007)
QacA/B	GCAGAAAGTGCAGAGTTCG CCAGTCCAATCATGCCTG	361 bp	Noguchi *et al*. (2005)
QacC/D	GCCATAAGTACTGAAGTTATTGGA GACTACGGTTGTTAAGACTAAACCT	195 bp	

Table 2. Temperature and time conditions of the primers during PCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Int1	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
Int3	95˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
bla_{TEM}	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
bla_{SHV}	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
QacED1	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
QacA/B	94˚C 5 min.	94˚C 30 sec	53˚C 40 sec	72˚C 40 sec	35	72˚C 10 min.
QacC/D	94˚C 5 min.	94˚C 30 sec.	53˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.

Table 3. Prevalence of resistance associated genes in the examined *E. coli* isolates (n.=10).

Types of genes	Genes	Results	
		Positive	%
Antibiotic resistance associated genes	bla_{TEM}	10	100%
	bla_{SHV}	6	60%
	Int1	10	100%
	Int3	2	20%
Disinfectant resistance associated gene	*QacED1*	8	80%
	QacA/B	2	20%
	QacCD	2	20%

β-lactamases resistance genes (bla_{TEM}, bla_{SHV}); Integron resistance genes (*Int1*, *Int3*). Quaternary ammonium compounds resistance genes (*QacED1*, *QacA/B*, *QacCD*).

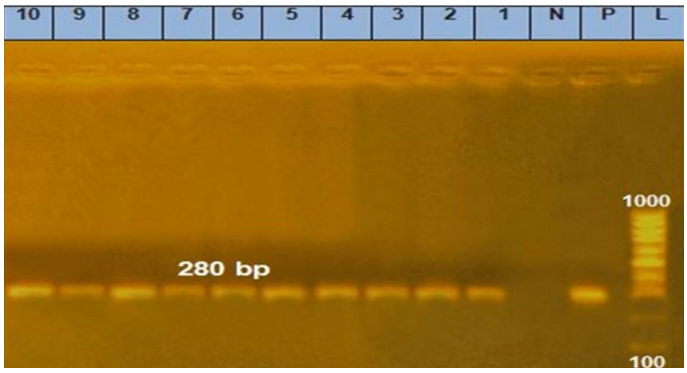

Fig. 4. PCR amplification of the 280bp fragment of *Int1* resistance gene from 10 *E. coli* isolates (1-10) Pos (control positive) Neg. (control negative).

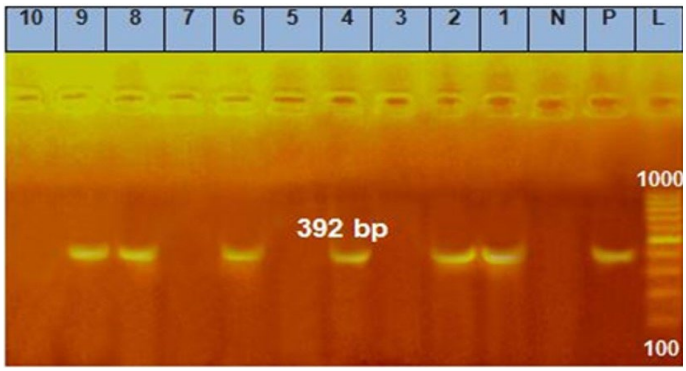

Fig. 1. PCR amplification of the 392bp fragment of bla_{SHV} resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

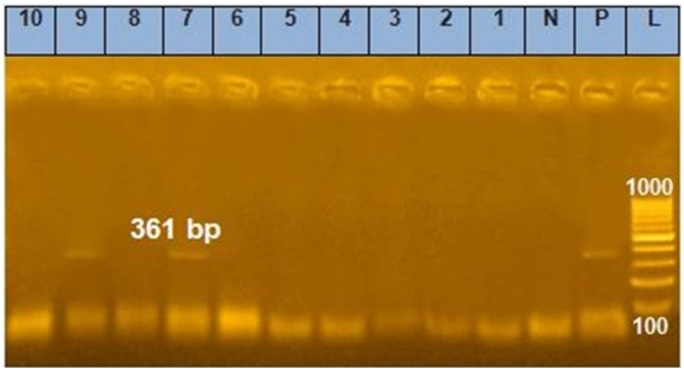

Fig. 5. PCR amplification of the 361bp fragment of *QacA/B* resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

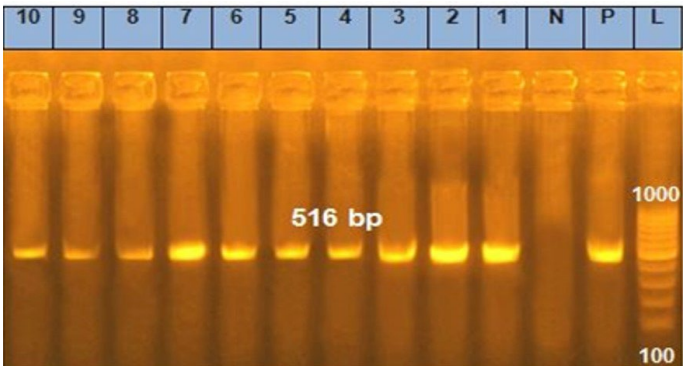

Fig. 2. PCR amplification of the 516bp fragment of bla_{TEM} resistance gene from 10 *E. coli* isolates (1-10), Pos (control positive), Neg. (control negative).

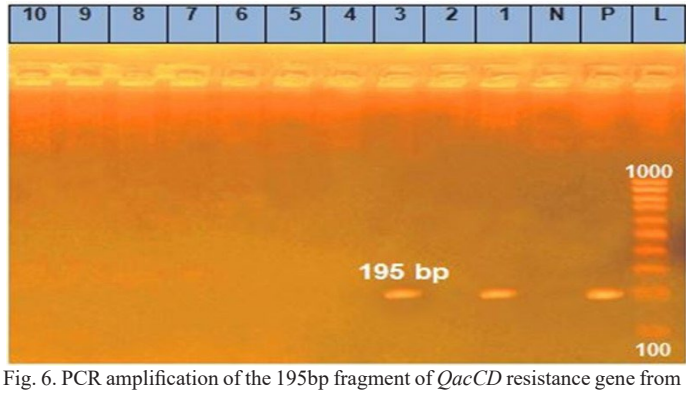

Fig. 6. PCR amplification of the 195bp fragment of *QacCD* resistance gene from 10 *E. coli* isolates (1-10) Pos. (control positive), Neg. (control negative).

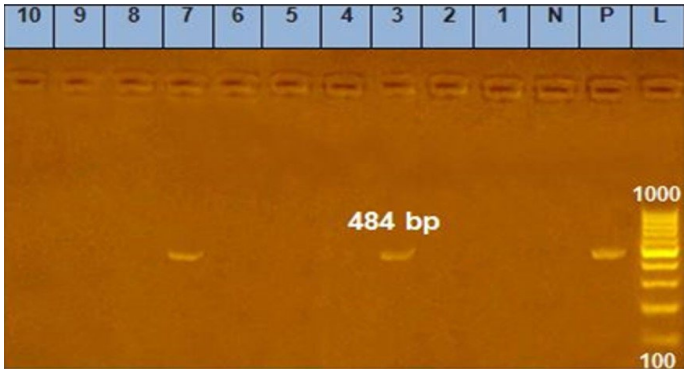

Fig. 3. PCR amplification of the 484bp fragment of *Int3* resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

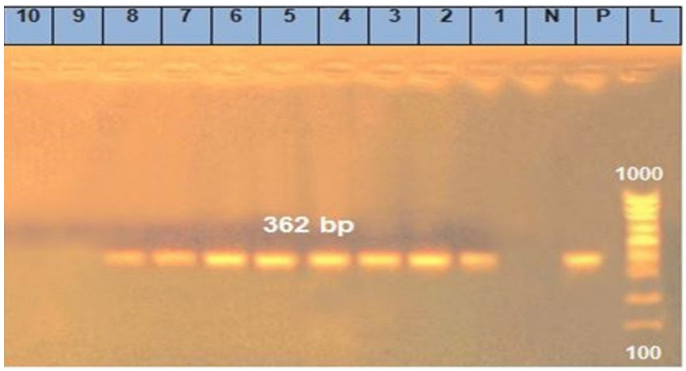

Fig. 7. PCR amplification of the 362bp fragment of *QacED1* resistance gene from 10 *E. coli* isolates (1-10), Pos. (control positive), Neg. (control negative).

DISCUSSION

Some of the most frequently used antibiotics, are β-lactam antibiotics which increase antibiotic resistance due to selective pressure (Beceiro *et al*., 2013). The production of an enzyme that hydrolyzes the antibiotic beta-lactam ring is one of the methods through which bacteria become resistant to antibiotics. High-powered new bacterial enzymes known as extended-spectrum lactamases are resistant to lactam antibiotics (Shaikh *et al*., 2015).

In the current work, PCR was applied on 10 Multidrug resistance *E. coli* (MDR) isolates to detect four antibiotic resistance associated gene and three disinfectant resistance associated gene PCR results for detecting ß-lactam resistant gene (bla_{TEM}) showed that 10/10 of isolates had a bla_{TEM} gene.

The results agreed with Salem *et al*. (2023) and El Seedy *et al*. (2019) who found that bla_{TEM} gene was the most prominent gene while Abdelaziz *et al*. (2022) observed that about 95% of *E. coli* isolates carried bla_{TEM} gene. 94% harbored bla_{TEM} gene that was recorded by Dhaouadia *et al*. (2020) and 93% of bla_{TEM} gene was recorded by Mona *et al*. (2023).

Class 1 integrons are widely distributed among Gram-negative bacteria. Clinical integrons are the most prevalent class of Integrons found in clinical isolates (Gillings., 2017). Also, 100% of multi drug resistance *E. coli* isolates had class I integron gene, this finding is matched with results shown by Ibrahim *et al*. (2019) who recorded that 97% of *E. coli* isolates had *Int1* gene while 72.7% had class I integron (Kalantari *et al*., 2021).

On the other hand, Dhaouadia *et al*. (2020) recorded only 18% of *E. coli* isolates haboured *Int1* gene, which is lower than the obtained result. The results revealed also about 60% of *E. coli* isolates had bla_{SHV} gene and only 20% of *E. coli* isolates had *Int3* gene.

This finding is matched with that reported by Salem *et al*. (2023) who documented that bla_{SHV} gene is the most predominant gene, meanwhile Mona *et al*. (2023) stated that 35.7% of the isolates had bla_{SHV} gene which is lower than the current results.

Quaternary ammonium compounds (QACs) are cationic surface active detergents that are mostly used in poultry farm due to their low relative toxicity, good antibacterial capabilities, non-irritating, non-corrosive, low toxicity, and effective in the presence of organic matter, As a result, it is the preferred disinfectant for incubators and hatching trays (Haynes *et al*., 2003). Quaternary ammonium compounds resistance genes are QacE and *QacED1*; *QacED1* is a mutant variant of qacE that is extensively distributed across Gram-negative bacteria and it is located on the 3′ conserved area of class 1 integrons, suggesting that it may be partially functional as a multidrug transporter (Kazama *et al*., 1999).

According to Quaternary ammonium disinfectant resistance associated gene (*QacED1*, *QacCD* and Qac A/B), 80% of *E. coli* isolates had *QacED1*, this result is matched with the record of Ibrahim *et al*. (2019) who found *QacED1* gene in 70.6% *E. coli* isolates. Higher result than that recorded in the present study was obtained by Enany *et al*. (2019) who observed that 100% of the isolates has *QacED1* gene. But only 20% of *E. coli* isolates in current study had *QacCD* and Qac A/B.

CONCLUSION

Multidrug-resistant pathotypes are frequently linked to several QACs resistance genes, proper administration of antibiotic and disinfectant should be applied.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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