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Virulence, Resistance Profile, Antimicrobial Resistance Genes of ESBLs, XDR *Escherichia coli* Isolated from Ducks

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Abstract

Ducks are possible carriers of zoonotic diseases to humans. Public health is impacted by the widespread dissemination of *Enterobacteriaceae* carrying extended-spectrum-lactamase (ESBL) genes. The prevalence of antimicrobial resistance in *Escherichia coli* isolated from Egyptian ducks, as well as the molecular characteristics of ESBLs to ESBLs genes and non-ESBLs genes, were studied.15% *E. coli* isolates were recovered from duck, and all of them were virulent as hemolytic and congo red positive. All ESBL-producing *E. coli* isolates were resistant to tetracycline, and nalidixic acid and 83.3% of isolates were also resistant to trimethoprim/sulfamethoxazole, penicillin, both ceftazidime, and cefotaxime. Recovered ESBL-producing *E. coli* strains harbored *qnrA*, *tetA*, *Sul1*, bla_{TEM}, bla_{CTX-M}, *aadA1*, bla_{OXA-1}, and bla_{SHV} antimicrobial-resistance genes with a prevalence of 100%, 100%,83.3%, 83.3%, 83.3%, 75%, 58.3%, and 41.6%, respectively. 33.3% of the ESBL-producing *E. coli* isolates were MDR, and 66.7% were recognized as XDR. The extension of ESBLs- *E. coli* threatens public health should be carefully monitored.

KEYWORDS
Antimicrobial resistance genes, *E. coli*, Hemolysis, MDR, XDR, ESBLs

INTRODUCTION

Ducks provide protein (meat and eggs) and sustenance for duck producers. (Adzitey *et al.*, 2012). *Escherichia coli* is the most frequent commensal Gram-negative rod bacteria and a food safety marker (Ramos *et al.*, 2020). Certain *E. coli* strains can harm humans and animals (Venkitanarayanan *et al.*, 2019). Septicemia, peritonitis, meningitis, diarrhea, and urinary tract infections are all signs of bacteria that cause extraintestinal and intestinal illnesses (Gholami-Ahangaran *et al.*, 2021). *E. coli* pathogenic strains are responsible for 30% of chicken deaths and a wide variety of disease syndromes (Radwan *et al.*, 2020). Antibiotic resistance could develop in bacteria unless they are misused and overused (Algammal *et al.*, 2022). Antibiotic resistance is a widespread issue with negative effects on the management of infectious illnesses (Algammal *et al.*, 2021). Beta-lactam antibiotics are the most frequently prescribed antibiotics for treating bacterial infections in both animals and humans due to their low toxicity and effective antimicrobial activity (Carvalho *et al.*, 2020). This bacteria's genetic flexibility and ability to adapt to different settings make it an ecologically important bioindicator of antibiotic resistance (Ramos *et al.*, 2020).

Human health is impacted by *E. coli* which produces extended-spectrum β-lactamase (ESBL). Resistance to first-, second- and third-generation cephalosporins (3GCs), penicillins, and monobactams are conferred by ESBLs, which are β -lactamase enzymes (Collis *et al.*, 2022) and are inhibited by lactamase inhibitors like clavulanic acid (Paterson and Bonomo, 2005). Numerous ARGs can be carried by plasmids with ESBL genes, leading to a multi-drug resistant trait (Collis *et al.*, 2022). Most of the broad-spectrum beta-lactamases are produced by *Enterobacteriaceae*, including *E. coli* (Samanta *et al.*, 2018). Because of the genes on plasmids that can span species boundaries, ESBLs that were initially found in *Klebsiella* species in the 1980s and later identified in *E. coli* and other *Enterobacteriaceae* are rapidly increasing in *E. coli* and other Gram-negative bacilli (Taru *et al.*, 2015). A majority of ESBLs are TEM, SHV, or CTX-M (Tola *et al.*, 2021). If strains isolated from poultry generate large amounts of beta-lactamases, there is a greater danger that these genes will spread to other microorganisms as well as human microorganisms (Gholami-Ahangaran *et al.*, 2021). This study investigated ducks' ESBL-producing *E. coli* strains, antibiotic resistance, ESBL genes, and non-ESBL genes.

MATERIALS AND METHODS

Ethics declaration

The study's animal care followed the recent ARRIVE guideline. The Animal Ethics Review Committee of Suez Canal University (AERC-SCU), Egypt, approved all experiments by trained scientists.

Bacterial analysis

Eighty duck samples were randomly collected from slaugh-

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terhouses and retail poultry shops in Portsaid governorate, Egypt. Gram staining, growth characteristics on culture media, and the results of biochemical tests were used to identify and isolate *E. coli*. (Mac Faddin, 1985).

Phenotypic virulence factors of retrieved isolates

A-Congo-red binding test

A Congo-red binding assay (Difco, USA) was implemented to evaluate the recovered isolates' in-vitro invasiveness and pathogenicity (Panigrahy and Yushen, 1990).

B-Hemolysis on blood

For the hemolysin test, the presumptive *E. coli* isolates were inoculated onto 5 % sheep blood agar and incubated overnight at 37 ºC (Quinn *et al*., 2011).

Phenotypic analysis of extended-spectrum beta-lactamase (ESBLs)

The presence of ESBL was identified through the application of a Double-disk synergy test (DDST) with cefotaxime (CTX), ceftazidime (CAZ), and amoxicillin-clavulanic acid (AMC) (Jarlier *et al*., 1988).

Antimicrobial susceptibility testing of E. coli

The antibiogram of *E. coli* was performed with nine tested antimicrobial agents (Penicillin (PEN,10 U), amoxicillin-clavulanic acid (AMC,20/10 µg), Ceftazidime (CAZ,30 µg), cefotaxime(30µg, CTX), tetracycline(TE,10µg), imipenem (IMP,10 µg), trimethoprim/sulphamethoxazole (19:1 µg, SXT), streptomycin (STR,10 µg), and nalidixic acid (ND,30 µg)) using the disc diffusion method (CLSI, 2018). According to Magiorakos *et al*. (2012), the phenotypic resistance patterns are divided into XDR and MDR.

Virulence and antimicrobial resistance genes revealed in retrieved E. coli using PCR

bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *Sul1*, *tetA*, *qnrA*, and *aadA1* antimicrobial resistance genes were detected using multiplex and uniplex PCR. According to the instructions provided with the QIAamp DNA Mini Kit (QIAGEN Sciences Inc., Germantown, Maryland, United States of America/Catalog No. ID 51326), bacterial DNA was extracted. Oligonucleotide sequences (provided by Thermo Fisher Scientific, USA), as well as the techniques for thermal cycling, are outlined in Table 1.

Statistical analysis

Chi-square analysis (P 0.05) and correlation analysis using the corrplot package in R-software were used to interpret the data.

RESULTS

The phenotypic features and the prevalence of E. coli

The isolates were typical *E. coli* morphology which lactose fermenters on MacCkoney agar and Metallic sheen colonies on EMB. All isolates tested negative for oxidase, H2S generation, citrate consumption, urease production, and Voges-Proskauer, but Methyl-red, catalase, indole, and nitrate reduction assays were positive. The prevalence of *E. coli* in the examined samples was 15 % (12/80). *E. coli* was highly isolated from the liver. Non-significant differences between different organs as illustrated in Table 2.

Phenotypic virulence of recovered E. coli

All recovered isolates were hemolytic on Blood agar and all tested isolates were positive for Congo-red binding.

Phenotypic analysis of ESBLs

Synergism with clavulanic acid and the presence of ESBL was indicated by a development of the inhibition zones of either cephalosporin discs or AMC. All isolates were ESBL-producing *E. coli*, as shown in Figure 1.

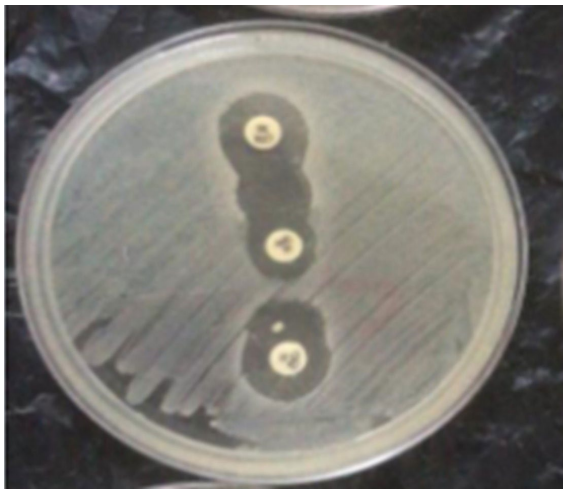

Figure 1. Detection of ESBLs production by double disc synergy test.

Antimicrobial susceptibility tests

Recovered ESBL isolates showed remarkable resistance to tetracycline, nalidixic acid, penicillin, trimethoprim-sulfamethoxazole, both ceftazidime and cefotaxime, streptomycin, and amoxicillin-clavulanic acid with a prevalence of 100%, 100%, 83.3%, 83.3%, 83.3%, 75 %, and 66.7%, respectively. Moreover, all recovered isolates exhibited sensitivity to carbapenem, as illustrated in Table 3. A non-significant statistical difference ($P < 0.05$) in the susceptibility of *E. coli* isolates to antimicrobials.

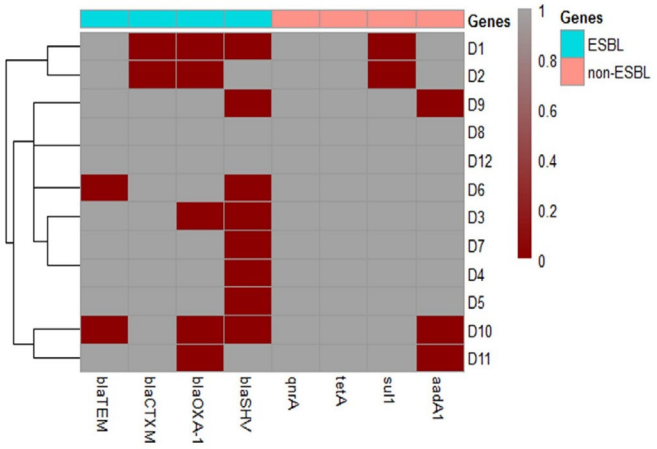

Figure 2. The collective distribution between phenotypic resistance, resistance patterns, ESBLs genes, and non-ESBLs genes.

Antimicrobial resistance genes of ESBL-producing E. coli

Recovered ESBL-producing *E. coli* strains harbored *qnrA*, *tetA*, *Sul1*, bla_{CTX-M}, bla_{TEM}, *aadA1*, bla_{OXA-1}, and bla_{SHV} antimicrobial-re-

sistance genes with a prevalence of 100%, 100%, 83.3%, 83.8%, 83.3%, 75%, 58.3%, and 41.6%, respectively, as illustrated in Table 4, and Figure 2. Statistically, a non-significant difference ($P < 0.05$) in the ESBL non-ESBL genes among the ESBL *E. coli* strains.

Phenotypic multidrug-resistance profiles and the distribution of ESBLs and non-ESBLs genes among ESBL E. coli isolates

33.3% (4/12) of the ESBL-producing *E. coli* isolates were recognized as MDR. Besides, 66.7% (8/12) of the ESBLs isolates were recognized as XDR as illustrated in Table 5. The correlation coefficient (r) was determined between tested antimicrobial agents and the antimicrobial resistance genes, as illustrated in Figure 3.

DISCUSSION

Duck meat is a very popular food in Egypt and worldwide which contains essential and non-essential amino acids in animal-based proteins (USDA⁄ARS, 2013). The current research aimed to assess the prevalence, antimicrobial-resistance profiles, hemolysis, phenotypic pathogenicity, and PCR-based detection of ESBLs genes, and non -ESBLs genes of emerging *E. coli* in ducks.

Eighty duck samples were examined using a bacteriological and biochemical analysis. Similar to Darwish *et al.* (2015), 15%

Table 1. List of used primers

	Genes	Oligonucleotides sequences	Amplified product (bp)	PCR conditions (35 cycles)			References
				Denaturation	Annealing	Extension	
Tetracycline	*tetA*	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576	94˚C 30 sec.	50˚C 40 sec	72˚C 45 sec	Randall *et al.* (2004)
Streptomycin	*aadA1*	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484	94˚C 30 sec	50-54˚C 40 sec	72˚C 45 sec	
Sulfonamide	*Sul1*	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433	94˚C 30 sec.	54˚C 40 sec	72˚C 45 sec.	Ibekwe *et al.* (2011)
ESBLs	bla_{CTX-M}	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAA YCAGCGG	593	94˚C 30 sec	54˚C 40 sec	72˚C 45 sec	Archambault *et al.* (2006)
	bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392	94˚C 30 sec.	54˚C 40 sec	72˚C 45 sec.	Colom *et al.* (2003)
	bla_{OXA-1}	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	564	94˚C 30 sec.	54˚C 40 sec	72˚C 45 sec.	Perez *et al.* (2007)
	bla_{TEM}	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800	94˚C 30 sec	54˚C 40 sec	72˚C 45 sec	
Fluoroquinolones	*qnrA*	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	94˚C 30 sec	55˚C 40 sec	72˚C 45 sec	Robicsek *et al.* (2006)

Table 2. Various types of obtained samples from ducks.

Type of samples	No. of recovered isolates	% of recovered isolates
Liver n= 20	5	41.6
Heart n= 20	3	25
Lung n= 20	2	16.7
Gizzard n= 20	2	16.7
Total n.= 80	12	100
Chi-square	2	
P value	0.5724^{NS}	

Table 3. Antibiotics-resistance phenotypes of ESBL-producing *E. coli*.

Antibiotic classes		Tested antibiotic	Interpretation					
			Sensitive		Intermediate		Resistance	
			N	%	*N*	%	*N*	%
ESBLs	Penicillins	Penicillin	-	-	2	16.7	10	83.3
		Amoxicillin-clavulanic acid	-	-	4	33.3	8	66.7
	Cephalosporins	Cefotaxime	1	8.3	1	8.3	10	83.3
		Ceftazidime	2	16.7	-	-	10	83.3
	Carbapenem	Imipenem	10	83.3	2	16.7	0	0
Aminoglycosides	Aminoglycosides	Streptomycin	3	25	-	-	9	75
Quinolones	Quinolones	Nalidixic acid	-	-	-	-	12	100
Tetracycline	Tetracycline	Tetracycline	-	-	-	-	12	100
Sulfonamide	Sulfonamides	Trimethoprim-Sulfamethoxazole	2	16.7	-	-	10	83.3
Chi square			9.8		16		11.56	
P value			0.2793^{NS}		0.04238^{NS}		0.1722^{NS}	

Table 4. Prevalence of ESBLs and non-ESBLs genes of ESBL producing *E. coli*.

	Genes type	*N*	%	*P* value
Non-ESBLs resistance genes	*tetA*	12	100	0.89^{NS}
	qnrA	12	100	
	Sul1	10	83.3	
	aadA1	9	75	
ESBLs resistance genes	bla_{TEM}	10	83.3	0.5222^{NS}
	bla_{CTX-M}	10	83.3	
	bla_{OXA-1}	7	58.3	
	bla_{SHV}	5	41.6	

Table 5. Collective distribution between phenotypic resistance, resistance patterns, ESBLs genes, and non-ESBLs genes.

Isolate	Phenotypic resistance	ESBLs genes	Non- ESBLs genes	Resistance pattern	MARI
1	TE, ND, P, STR	bla_{TEM}	*tet*A, *qnr*A, *aad*A1	MDR	0.44
2	TE, ND, P, STR	bla_{TEM}, bla_{SHV}	*tet*A, *qnr*A, *aad*A1	MDR	0.44
3	SXT, TE, ND, P, STR, CAZ, CTX, AMC	bla_{TEM}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.88
4	SXT, TE, ND, P, STR CAZ, CTX, AMC	bla_{TEM}, bla_{OXA1}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.88
5	SXT, TE, ND, P, STR, AMC, CAZ, CTX,	bla_{TEM}, bla_{OXA1}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.66
6	SXT, TE, ND, STR, CAZ, CTX, AMC	bla_{OXA1}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.77
7	SXT, TE, ND, P, STR CAZ, CTX, AMC	bla_{TEM}, bla_{OXA1}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.88
8	SXT, TE, ND, P, STR CAZ, CTX, AMC	bla_{TEM}, bla_{OXA1}, bla_{SHV}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.88
9	SXT, TE, ND, P, CAZ, CTX, AMC	bla_{TEM}, bla_{OXA1}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A	XDR	0.77
10	SXT, TE, ND, CAZ, CTX	bla_{CTX-M}	*tet*A, *sul*1, *qnr*A	MDR	0.55
11	SXT, TE, ND, P, CAZ, CTX	bla_{TEM}, bla_{SHV}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A	MDR	0.66
12	SXT, TE, ND, P, STR CAZ, CTX, AMC	bla_{TEM}, bla_{OXA1}, bla_{SHV}, bla_{CTX-M}	*tet*A, *sul*1, *qnr*A, *aad*A1	XDR	0.88

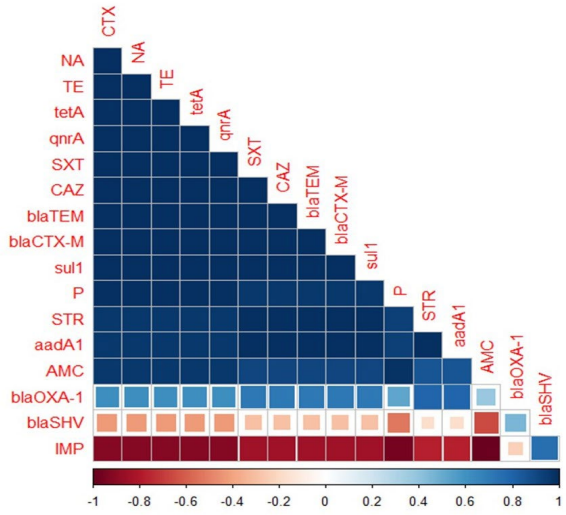

Figure 3. The Heat-map illustrates the correlation coefficient (r) among different tested antimicrobial agents, ESBLs genes, and non-ESBL genes.

of duck samples tested positive for *E. coli*. Among the various organs examined, the liver appeared to have the greatest per centage of *E. coli* isolates (41.6%), followed by the heart (25%), the gizzard (16.7%), and the lungs (16.7%). That the bacteria have moved from the intestines to the liver is one explanation for the high concentration of *E. coli* (Darwish *et al*., 2015). Egypt and other developing nations eat offal (liver, gizzard, and heart) as a fast meal since it is inexpensive, easy to produce, and an excellent source of proteins (Hassanin *et al*., 2017). All of the ESBL-*E. coli* tested in this research were hemolytic and congo red positive, indicating pathogenicity, Sharma *et al*. (2006) reported that the congo red dye agar test may distinguish invasive from non-invasive *E. coli* in poultry. In animal models, *E. coli* pathogenicity depends on cytolysin which is a pore-forming cytolytic toxin (Tzokov *et al*., 2006).

The bacteria were resistant to tetracycline, quinolones, penicillin, amoxicillin-clavulanic acid, cephalosporin, and sulfamethoxazole-trimethoprim similar to researchers who identified ESBL-producing *E. coli* in ducks in Egypt (Darwish *et al*., 2015), chicken farms in Egypt (Badr *et al*., 2022), and broiler farms in the Philippines (Gundran *et al*., 2019). In Korea, ESBL-producing *E. coli* was resistant to ampicillin, amoxicillin, and sulfamethoxazole-trimethoprim (Seo *et al*., 2018). 33.3% (4/12) of ESBL-producing *E. coli* samples in this study were MDR and 8/12 (66.7%) ESBL strains were XDR. In 2021, Aworh *et al*. (2021) identified 34.7% of *E. coli* samples were MDR, while Sonola *et al*. (2021) reported 27.7% of MDR *E. coli*, Iqbal *et al*. (2021) identified 92% (58/63) of samples were MDR and XDR pathogens threaten human health (Karaiskos and Giamarellou, 2014).

The ESBL resistance genes bla_{CTX-M} and bla_{TEM} (83.3%) were the most prevalent, followed by bla_{OXA-1} (58.3%) and bla_{SHV} (46.1%), according to PCR results used to validate the ESBL resistance genes. A new Japanese study found that CTX-M and TEM-producing ESBLs are common in agricultural and domestic animals (Ejaz *et al*., 2021). *Enterobacteriaceae* isolates from healthy Egyptian hens have ESBL-resistant genes bla_{TEM}, bla_{SHV}, and bla_{CMY} (Ahmed and Shimamoto, 2013). Another study found that ESBL-producing *E. coli* isolates from offal samples from 20 Egyptian poultry farms exhibited high bla_{CTX-M} gene concentrations (El-Shazly *et al*., 2017). ESBL-producing organisms also resist quinolones, aminoglycosides, tetracyclines, chloramphenicol, and trimethoprim. Plasmids, transposons, and integrons with many resistant genes can cause multidrug resistance (Machado *et al*., 2005). In this study, tetracycline resistance was highly prevalent in *E. coli* strain isolates, which is consistent with Amer, *et al*. (2018). The high rate of tetracycline resistance is due to its long-term therapeutic and preventative use as a first-line antibiotic. The *tetA* gene was frequently discovered in the MDR and XDR *E. coli* isolates in this research matched Algammal *et al*. (2020) in livestock and

Jurado-Rabadán *et al*. (2014) in Spanish pigs. This research successfully identified the *qnrA* gene. This protein blocks the binding of quinolones to DNA gyrase (Algammal *et al*., 2022). Variations and aminoglycoside nucleotidyltransferase make Gram-negative bacteria resistant to gentamicin, tobramycin, and streptomycin (Vuthy *et al*., 2017). Sulfonamide-resistant DHPS alternative alleles have been developed (Yun *et al*., 2012).

CONCLUSION

This study's findings add to the evidence that antibiotic resistance is widespread in Egypt and that the studied duck samples herein may be a major source of exposure and dissemination of PDR, XDR, MDR, ESBL-producing, and virulent *E. coli*.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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