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Molecular Detection of Virulence and Antibiotic Resistance Genes in Shiga Toxin-Producing *Escherichia coli* (STEC) Isolated from Sausage Meat in Egypt

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ABSTRACT

Escherichia coli (*E. coli*) is increasingly recognized as a pathogen that causes disease in humans and animals. Among its harmful strains, Verocytotoxigenic *E. coli* (VTEC), or Shiga toxin-producing *E. coli* (STEC), is a major concern due to its ability to spread through various foods and produce potent toxins. This study aimed to screen the serotypes and identify genes responsible for virulence and antibiotic resistance in STEC strains recovered from sausage meat in Egypt. To achieve this, 100 samples of sausage sandwiches were collected from the Giza and Cairo Governorates, Egypt. *E. coli* was isolated at a prevalence rate of 16%. The antibiotic resistance rate showed the highest rate at 100% for ampicillin and erythromycin. Serotyping of 16 *E. coli* isolates revealed that the most prevalent serotypes were O125:K70 (5/16, 31.25%), O118: K- (4/16, 25%), and O86:K61 (2/16, 12.5%). PCR analysis of genes associated with virulence in all *E. coli* isolates showed that the *stx1* and *stx2* genes were positive in 31.25% and 93.75% of the isolates, respectively, while the *iss* gene was positive in 50% of the isolates. In contrast, the genes *eae*A, *ast*A, and *pap*C were negative. PCR testing for antibiotic resistance genes revealed that the *tet*A gene was detected in 81.25%, while the *sul1* gene positivity rate was 75%. In contrast, the *aac(3)-IV*, *dfrA1*, and *mrc1* genes were all negative. The results of our study emphasize the need for ongoing monitoring and periodic diagnosis of STEC strains, as well as improved hygiene practices to protect public health.

Key words: *Escherichia coli*, Shiga toxin-producing *E. coli* (STEC), Virulence genes, Antibiotic resistance genes, Ready-to-eat meat.

INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative coliform bacterium that is widespread in different environments and includes several groups that can cause diseases in both humans and animals (Allocati et al. 2013). *E. coli* consists of hundreds of strains that are serologically classified according to surface antigens, including 173 somatic (O), 80 capsular (K), 56 flagellar (H) and an unspecified number of fimbrial (F) types (Sarowska et al. 2019; Sora et al. 2021). *E. coli* includes 160 pathotypes associated with gastrointestinal infections, surgical site infections, hospital-acquired pneumonia, and meningitis (Sarowska et al. 2019).

Diarrheagenic *Escherichia coli* (DEC) is an important bacterial pathogen responsible for causing gastroenteritis globally. The World Health Organization reports that foodborne bacterial diseases are responsible for over 300 million cases and approximately 200,000 deaths annually worldwide (Collins et al. 2024). Based on the presence of specific virulence traits, five DEC pathotypes have been well defined and are directly associated with disease progression. The main DEC are classified as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and Shiga toxin-producing *E. coli* (STEC) (Lee et al. 2023; Collins et al. 2024).

The spread of STEC is considered a significant health concern due to its potential to result in major illnesses, such as hemorrhagic colitis and hemolytic uremic syndrome, especially among young children and older adults. Ruminants are the primary natural reservoir for STEC as a zoonotic pathogen. The large number of outbreaks worldwide confirms the significance of these pathogens and emphasizes the necessity for obligatory disease reporting and collaboration among laboratories both domestically and internationally. Most severe cases worldwide are attributed to strains belonging to the O157, O26, O111, O103, and O145 serogroups. However, with improved detection and classification techniques, additional

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serogroups are identified more frequently, but assessment of pathogenicity increasingly relies on identifying the origins of virulence genes rather than phenotypic markers of the serogroup (Koutsoumanis et al. 2020).

Although the primary source of STEC is cattle, other ruminants, like sheep and goats, also contribute significantly, albeit to a lesser extent. STEC can colonize the intestine of these animals without causing symptoms. Humans can become infected through the consumption of contaminated food or water, exposure to animal waste, direct interaction with animals, or even person-to-person transmission (S¨oderlund et al. 2012).

To prevent food contamination with STEC, it is essential to apply good agricultural practices, strict hygiene measures and adherence to safe procedures across all stages of food production, processing, and distribution (FAO and WHO 2019; FAO and WHO 2022).

The pathogenicity of STEC primarily results from the production of Shiga toxins (Stx), which are encoded by the *stx* genes and exist in two principal forms: Stx1 and Stx2. These toxins are classified into specific subtypes according to a defined classification system, along with several new forms identified in recent years (EFSA 2020; Gill et al. 2022).

The health risks posed by foodborne pathogens through food poisoning are not the only serious threat; the transmission of antibiotic resistance is also a major concern. Food animals, inevitably, contribute significantly to the continued expansion of this challenge because they are the principal source of animal protein in the food supply. Bacterial antimicrobial resistance (AMR) develops when bacteria go through genetic changes that reduce the effectiveness of antibiotics. Human factors such as the improper administration of antibiotics in both human and veterinary healthcare, as well as inadequate hygienic conditions and practices in healthcare settings or in the food chain, all contribute to the spread of resistant microbes (European Union 2017).

The use of antimicrobial therapies for treating STEC infections has become a debated issue, as these drugs can cause bacterial cell lysis, leading to the liberation of Shiga toxins, which potentially increase the activation of *stx* genes within the host (Kakoullis et al. 2019).

Governmental and official surveys are less common in Egypt than they are in developed countries, so research is constantly required to provide information on the microbiological safety of meat products. In addition, recent high outbreaks and contamination rates of priority pathogens, specifically *Escherichia coli* and *Salmonella*, have been linked to ready-to-eat foods (Sabeq et al. 2022), including kebab meat (ECDC 2023) and ground beef (FSIS 2022).

Sausages are one of the most widely produced meat products and contribute significantly to the economy in many countries worldwide (Lonergan et al. 2019).

Therefore, this current study aimed to evaluate the presence of Shiga toxin *Escherichia coli* (STEC) as foodborne pathogens in ready-to-eat sausage meat and to detect genes associated with virulence and antibiotic resistance in the isolates to aid in infection control.

MATERIALS AND METHODS

Study period and location

The study was conducted from 2023 to 2024 at the National Research Center. Sample processing took place at the Microbiology and Immunology Department, Veterinary Research Institute, National Research Centre, Egypt.

Samples

A total of 100 ready-to-eat sausage sandwiches were collected from Giza and Cairo Governorates, Egypt. The samples were properly packaged, numbered, and transported in an icebox to the laboratory for further analysis.

Bacterial isolation and identification

Samples were examined under sterile conditions. Each sample was surface sterilized before collection. Then, 25 grams of each food sample were mixed with 225mL of modified tryptic soy broth (TSB) (Oxoid, UK) for 2 minutes and followed by incubation at 37°C for 24 hours. Samples were first cultured on MacConkey agar (Oxoid, UK) and SHIBAM plates prepared in the lab (Feng et al. 2011). After incubating at 37°C for 18-24 hours, colonies with characteristic *E. coli* appearance were picked and purified on eosin methylene blue (EMB) agar (Oxoid, UK). Suspected *E. coli* isolates were then identified using a series of biochemical tests, such as methyl red, indole, Voges-Proskauer, oxidase, citrate, urease, and triple sugar iron tests, as mentioned by Liu et al. (2017).

Serological typing of *E. coli* isolates

The isolates were identified by serotyping at the Animal Health Research Institute, Giza, Egypt, using Sifin polyvalent and monovalent antisera specific for *E. coli* (Berlin, Germany). Serotyping was done by agglutination according to Edwards and Ewing (1972).

Phenotypic detection of antibiotic resistance patterns

The susceptibility of the isolates to antibiotics was evaluated using the disk diffusion technique against the most important antibiotics: ampicillin (AMP; 10µg), erythromycin (E; 15µg), tetracycline (TET; 30µg), ciprofloxacin (CIP; 5µg), tobramycin (TOB; 10µg), colistin (CL; 10µg), gentamicin (GEN; 10µg), cefotaxime (CTX; 30µg), chloramphenicol (C; 30µg), and trimethoprim-sulfamethoxazole (COT; 1.25/23.75µg).

Each strain was streaked on Mueller-Hinton agar, and antibiotic discs were applied. The plates were inverted and kept at 37°C for 18 hours for incubation. The diameters of the inhibition zones were measured and recorded by classifying the inhibition zone diameter as sensitive, intermediate, or resistant according to criteria established by CLSI 2020.

DNA extraction

The GF-1 Bacterial DNA Extraction Kit (Cat. No. GF-BA-100, Vivantis Technologies, Malaysia) was used to extract DNA from bacterial cultures following the manufacturer's recommendations.

Confirmation of isolated *E. coli* using conventional polymerase chain reaction (pcr)

The extracted DNA was subjected to amplification using an *E. coli*-specific primer targeting the *phoA* gene, which encodes for *E. coli* (Yu and Thong 2009; Aliyu et al. 2016).

Molecular detection of virulence and antibiotic resistance genes in *E. coli* isolates using pcr

PCR was performed on the *E. coli* isolates to screen for specific virulence genes, including *stx*1, *stx*2, *pap*C, *iss*, *eae*A, and *ast*A. Additionally, all isolates were examined for the presence of antimicrobial resistance genes such as *tet*A, *dfr*A, *aac* (3)-IV, *mcr*A1, and *Sul*1. The primers used for amplification, along with their corresponding PCR product sizes, are provided in Table 1.

PCR amplification was performed using a SimpliAmp™ Thermal Cycler (Cat. No. A24811, Applied Biosystems, USA) with a final reaction volume of 25µL. Each mixture contained 12.5µL of 2× MyTaq™ Red Mix Master Mix (Cat. BIO-25043, Meridian Bioscience, UK), 1µL (10µM) of each primer, 1µL of target DNA, and 9.5µL of double-distilled water (DDW). The resulting PCR products were resolved on a 1.5% agarose gel and subsequently photographed and analyzed using the InGenius3 gel documentation system (Syngene, UK). The thermal cycling conditions are provided in Table 2.

RESULTS

E. coli isolation and identification

Suspected E. coli colonies appeared pink to dark pink on MacConkey agar. Gram staining followed by microscopic

Table 1: Primers Used in This Study

Gene	Primer	Nucleotide sequence (50—30)	Amplicon Size (bp)	References
*Pho*A	*PhoA* F	CGATTCTGGAAATGGCAAAAG	720	Hu et al. (2011)
	PhoA R	CGTGATCAGCGGTGACTATGAC		
*sxt*1	*sxt*1F	CTGGATTTAATGTCGCATAGTG	150	Guion et al. (2008)
	*sxt*1R	AGAACGCCCACTGAGATCATC		
*sxt*2	*sxt*2F	GGCACTGTCTGAAACTGCTCC	255	Guion et al. (2008)
	*sxt*2R	TCGCCAGTTATCTGACATTCTG		
*Pap*C	*Pap*C F	GTGGCAGTATGAGTAATGACCGTTA	200	López-Banda et al. (2014)
	*Pap*C R	ATATCCTTTCTGCAGGGATGCAATA		
Iss	*Iss* F	ATGTTATTTTCTGCCGCTCTG	266	Yaguchi et al. (2007)
	Iss R	CTATTGTGAGCAATATACCC		
*eae*A	*eae*A F	ATGCTTAGTGCTGGTTTAGG	248	Bisi-Johnson et al. (2011)
	*eae*A R	GCCTTCATCATTTCGCTTTC		
*ast*A	*ast*A F	CCATCAACACAG TAT ATCCGA	111	Yamamoto and Echeverria (1996)
	*ast*A R	GGTCGCGAGTGACGGCTTTGT		
*tet*A	*tet*A F	GGTTCACTCGAACGACGTCA	577	Randall et al. (2004)
	*tet*A R	CTGTCCGACAAGTTGCATGA		
mcr 1	*mcr* 1 F	CGGTCAGTCCGTTTGTTC	308	Liu et al. (2016)
	mcr 1 R	CTTGGTCGGTCTGTAGGG		
*dfr*A1	*dfr*A1 F	GGAGTGCCAAAGGTGAACAG	474	Sáenz et al. (2004)
	*dfr*A1 R	GAGGCGAAGTCTTGGGTAAAAC		
aac(3)-IV	*aac*(3)-IV F	CTTCAGGATGGCAAGTTGGT	286	Van et al. *(*2008)
	aac(3)-IV R	TCATCTCGTTCTCCGCTCAT		
Sul 1	*Sul* 1 F	TTCGGCATTCTGAATCTCAC	822	Van et al. (2008)
	Sul 1 R	ATGATCTAACCCTCGGTCTC		

Table 2: Cycling conditions for the molecular detection of all genes screened in this study

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Number of cycles
PhoA	94°C	94°C	58°C	72°C	72°C	35
	5min	30sec	45sec	45sec	10min	
*sxt*1	95°C	95°C	50°C	72°C	72°C	40
	5min	45sec	45sec	45sec	10min	
*sxt*2	95°C	95°C	50°C	72°C	72°C	40
	5min	45sec	45sec	45sec	10min	
PapC	95°C	94°C	58.2°C	72°C	72°C	40
	5min	30sec	30sec	40sec	8min	
Iss	94°C	94°C	54°C	72°C	72°C	35
	5min	30sec	45sec	45sec	10min	
*eae*A	94˚C	94°C	51°C	72°C	72°C	35
	5min	30sec	30sec	45 sec	8min	
*ast*A	94°C	94°C	55°C	72°C	72°C	35
	5min	30sec	30sec	45sec	10min	
*tet*A	94°C	94°C	55°C	72°C	72°C	35
	5min	60sec	60sec	1min	10min	
*mcr*1	94°C	94°C	58°C	72°C	72°C	35
	5min	30sec	30sec	1min	10min	
*dfr*A1	94°C	94°C	55°C	72°C	72°C	35
	5min	30sec	30sec	45sec	10min	
aac(3)-IV	94°C	94°C	55°C	72°C	72°C	35
	5min	60sec	60sec	1min	8min	
Sul 1	94°C	94°C	60°C	72°C	72°C	35
	5min	30sec	40sec	45sec	10min	

examination confirmed the presence of Gram-negative bacilli. Biochemical tests showed the following results: positive for methyl red and indole, negative for Voges-Proskauer, oxidase, citrate, and urease, and acid production with gas but without hydrogen sulfide formation on TSI agar. Based on these results, the isolates were identified as E. coli. Out of 100 samples of ready-to-eat sausage sandwiches, 16 E. coli isolates were obtained, corresponding to an incidence rate of 16%.

E. coli serotyping

Sixteen *E. coli* isolates were examined for serotyping of the biochemically identified strains. The most predominant serotypes were as follows: O125:K70 (5/16, 31.25%), O118: K- (4/16, 25%), O86:K61 (2/16, 12.5%), O44:K74 (1/16, 6.25%), O44:K79 (1/16, 6.25%), O55:K59 (1/16, 6.25%), O142:K86 (1/16, 6.25%), O158: K- (1/16, 6.25%).

Antimicrobial resistance

The greatest level of antibiotic resistance was recorded against ampicillin and erythromycin (100%), followed by cefotaxime (81.25%), tobramycin (62.5%), and colistin (56.25%). Resistance to tetracycline and trimethoprim-sulfamethoxazole was recorded at 43.75%, while ciprofloxacin resistance was 25%. The frequency of resistance to chloramphenicol and gentamycin was the lowest at 12.5%.

Regarding antibiotic sensitivity, the highest rate was observed for gentamycin (81.25%), followed by chloramphenicol (68.75%), ciprofloxacin (62.5%), tetracycline (56.25%), trimethoprim-sulfamethoxazole (50%), tobramycin (37.5%), and cefotaxime (6.25%).

Molecular confirmation of isolated *E. coli* using pcr

All 16 *E. coli* isolates showed positive amplification of a 720bp fragment, as shown in Fig. 1.

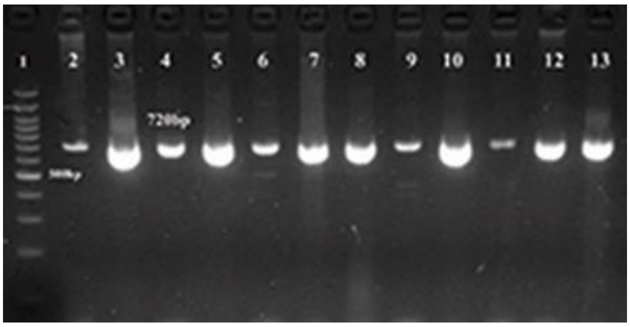

Fig. 1: Confirmation of *E. coli pho*A gene (720bp) through PCR. Lane 1 (100bp) marker, Lanes 2-13: PCR positive isolates.

Molecular detection of virulence genes

Screening of all 16 *E. coli* isolates for the *stx*1 (150bp) and *stx*2 (255bp) genes resulted in 5 (31.25%) and 15 (93.75%) positivity, respectively. The *iss* gene (266bp) was detected in 8 (50%) of the isolates. However, all isolates tested were negative for *eaeA*, *astA*, and *papC* genes. as shown in Fig. 2, 3, and 4.

Molecular detection of antibiotic resistance genes

Screening of all 16 *E. coli* isolates for the *tetA* gene (577bp) and the *sul1* gene (822bp) showed positivity rates of 13 (81.25%) and 12 (75%,) respectively. However, all isolates tested negative for the *mrc1*, *aac(3)-IV*, and *dfrA1* genes, as shown in Fig. 5 and 6.

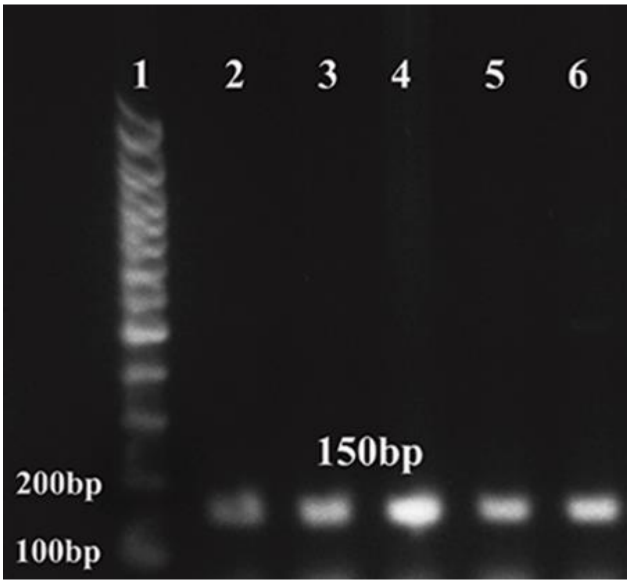

Fig. 2: Confirmation of *E. coli stx*1 gene (150bp) through PCR. Lane 1 (100bp) marker, Lanes 2-6: PCR positive isolates.

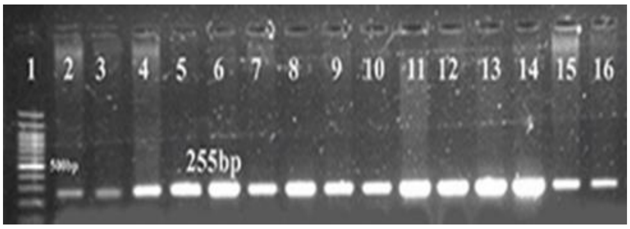

Fig. 3: Confirmation of *E.coli stx2* gene (255bp) through PCR. Lane 1 (100bp) marker, Lanes 2-16: PCR positive isolates.

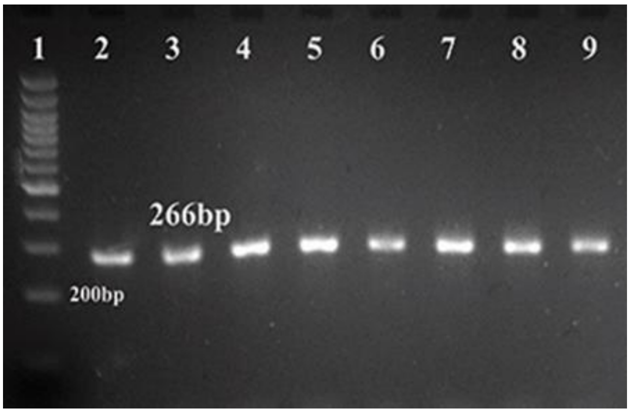

Fig. 4: Confirmation of *E. coli iss* gene (266bp). Lane 1 (100bp) marker, Lanes 2-9: PCR positive isolates.

DISCUSSION

Escherichia coli is a serious foodborne pathogen representing a significant concern regarding health standards and the safety of food supplies worldwide. Food microbiological standards identify disease-causing *E. coli* in food products. Due to the high risk of illness, a "zero tolerance" policy is applied for the majority of ready-to-eat food items. Non-ready-to-eat foods may contain a limited

E. coli, especially from STEC strains, exposing consumers to potential risks that lead to severe health problems such as hemolytic uremic syndrome (HUS) and even death (Alhadlaq et al. 2024).

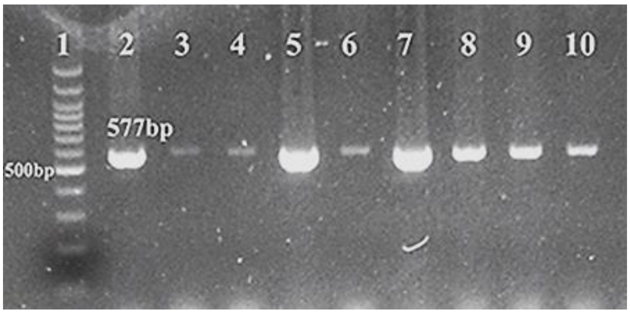

Fig. 5: Confirmation of *E. coli tet*A gene (577bp). Lane 1 (100bp) marker, Lanes 2-10: PCR positive isolates.

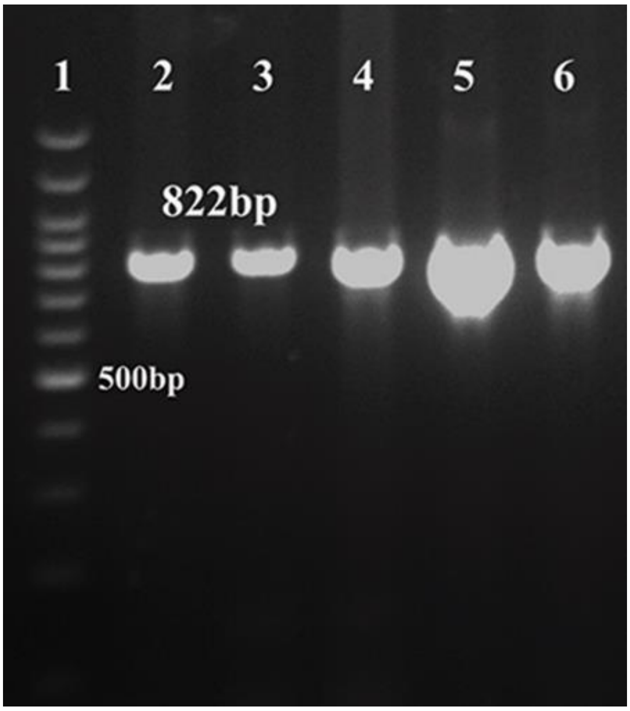

Fig. 6: Confirmation of *E. coli sul*1 gene (822bp). Lane 1 (100bp) marker, Lanes 2-6: PCR positive isolates.

In this study, an outbreak of *E. coli* was investigated by analyzing 100 samples of ready-to-eat sausage sandwiches. A total of 16 *E. coli* isolates were identified, representing an incidence rate of 16%. Our results are lower in incidence than those found by Masood et al. (2025), who found that (hawawshi and kofta) was prevalent in raw meat products (hamburgers and sausages) at a rate of 57.7%. However, since ready-to-eat meat products will not undergo further processing, the risk associated with receiving such contaminated ready-to-eat products would be significantly higher (Masood et al. 2025).

In a related study, Abd El-Tawab et al. (2019) found that among 175 meat and meat product samples, the occurrence of *E. coli* was 6.3%, with 8.6% in kofta and sausage each, 5.7% in fresh beef and beef burgers each, and 2.9% in luncheon meat. Additionally, Worku et al. (2022) recorded a reduced occurrence of *E. coli* in minced meat (2.5%).

Adzitey et al. (2021) reported *E. coli* contamination levels of 80% in raw beef and 50% in ready-to-eat beef. These differences may be attributed to differences in industrial procedures, exposure times, and handling methods.

Since its discovery in 1982, more than 39,787 cases of *E. coli* infection have been documented, including 1,343 epidemic cases. The Americas reported the most cases, followed by Africa, Asia, and Europe. The main serotypes associated with these outbreaks are O157, together with the six most frequently detected serogroups (O26, O45, O103, O111, O121, and O145), as well as other serotypes such as O55, O80, O101, O104, O116, O165, O174, and O183 (Alhadlaq et al. 2024).

In this study, the most predominant serotypes were O125:K70 (5/16, 31.25%), O118: K- (4/16, 25%), O86:K61 (2/16, 12.5%), O44:K74 (1/16, 6.25%), O44:K79 (1/16, 6.25%), O55:K59 (1/16, 6.25%), O142:K86 (1/16, 6.25%), and O158:K- (1/16, 6.25%).

Masood et al. (2025) revealed that all 26 *E. coli* isolates isolated from raw and ready-to-eat (RTE) meat products were of five different serotypes (O26, O55, O111:H4, O124, O126), three pathotypes: enterohemorrhagic (EPEC) 34.6%, enteropathogenic (EPEC) 46.15%, and enteroinvasive (EIEC) 19.23%. The highest prevalence of serotype was O26 (34.6%) and distribution in the four products evaluated, notably RTE. The most similar results were in KSA, where 120 *E. coli* isolates from food had a prevalence of 22.22% and included O26: K60, O55: K59, O111: K58, O126: K58, O128: K67, O86: K61, and O157: H7 (Hemeg 2018).

Furthermore, serotyping of *E. coli* isolated from raw meat samples such as raw minced beef and frozen beef liver and from meat products including kofta and processed beef sausage identified the presence of serotypes O26:H11, O91:H21, O124, and O128:H2 (Abdel-Atty et al. 2023).

Antimicrobials are used to treat and prevent disease as well as to promote livestock growth. These practices contribute to the development of drug-resistant microorganisms in food-producing animals. These resistant strains can threaten human health by contaminating animal products during slaughter and subsequent processing (Zhu et al. 2019).

E. coli ranked first among the top six pathogens responsible for resistance-related deaths. In 2019, antimicrobial resistance (AMR) was associated with approximately 929,000 deaths caused by six major AMR pathogens, and a total of 3.57 million deaths were linked to AMR overall. Moreover, six additional pathogen–drug combinations, including resistance to third-generation cephalosporins in *E. coli* and resistance to fluoroquinolones in *E. coli*, resulted in approximately 50-100 thousand deaths (Courtenay et al. 2019; Murray et al. 2022).

In the present study, ampicillin and erythromycin showed the highest resistance rates, with all isolates (100%), followed by cefotaxime (81.25%), tobramycin (62.5%), and colistin (56.25%). Resistance to tetracycline and trimethoprim-sulfamethoxazole was recorded at 43.75%, while ciprofloxacin resistance was 25%. The frequency of resistance to chloramphenicol and gentamycin was the lowest at 12.5%. The highest rate of antibiotic sensitivity was observed for gentamycin (81.25%), followed by chloramphenicol (68.75%), ciprofloxacin (62.5%), tetracycline (56.25%), trimethoprim-sulfamethoxazole (50%), tobramycin (37.5%), and cefotaxime (6.25%).

Regarding this, Moawad et al. (2017) reported that *E. coli* isolates were resistant to ampicillin (71.4%), trimethoprim/sulfamethoxazole (61.9%), and amoxicillin-clavulanic acid (61.9%). However, the isolates showed sensitivity to ciprofloxacin and ceftriaxone at 47.6% and 42.8%, respectively. Furthermore, ampicillin resistance was observed in 50% of *E. coli* isolates collected from beef (Messele et al. 2017).

Abdel-Atty et al. (2023) reported that all *E. coli* strains isolated from meat and meat products exhibited complete resistance (100%) to penicillin, amoxyciliin/ciavulnic acid, ampicillin/sulbactam, ceftazidime, erythromycin, ceftriaxone, clindamycin, and cefotaxime (100%). Furthermore, the isolated strains showed high susceptibility to tetracycline (87.5%). In contrast, they exhibited the highest resistance to gentamicin and linezolid (87.5%), followed by sulfamethizole/trimethoprim, imipenem, meropenem, and ciprofloxacin (62.5%).

Furthermore, Abd El-Tawab et al. (2019) demonstrated high resistance to amoxicillin and ampicillin (81.8%), streptomycin (72.7%), and erythromycin (63.6%) in *E. coli* isolates recovered from meat and meat products. Additionally, Zhao et al. (2012) showed that approximately half (50.3%) of *E. coli* strains collected from different types of commercially sold meat in the United States exhibited resistance to tetracycline.

STEC primarily causes disease by producing Shiga-like toxins, divided into two primary types, Stx1 and Stx2, which are differentiated into two main types, Stx1 and Stx2 (coded by the *stx*1 and *stx*2 genes). Moreover, STEC is highly infectious and carries specific virulence genes associated with protein secretion systems, adhesins, and various toxins (Amézquita-López et al. 2018).

Studies have indicated that the *stx*2 gene, rather than *stx*1, especially when combined with the *eae* gene, is more associated with serious human diseases (FAO and WHO 2019; EFSA 2020). Among the *stx*2 variants, the *stx*2a subtype is the one most connected to human disease

(Dallman et al. 2015; Byrne et al. 2018; FAO and WHO 2019).

This investigation revealed that all 16 *E. coli* isolates for the *stx*1 (150bp) and *stx*2 (255bp) genes resulted in 5 (31.25%) and 15 (93.75%) positivity, respectively. The *iss* gene (266bp) was detected in 8 (50%) of the isolates. However, all isolates tested negative for *eae*A, *ast*A, and *pap*C genes.

Our findings agree with those of Olaitan et al. (2025) on the high incidence of *stx*2 gene in *E. coli* isolates. The virulence genes *stx1*, *stx2*, *eaeA*, and *hlyA* were evaluated in beef samples from pathogenic *E. coli* strains in Nigeria. Of the molecularly confirmed isolates, 11.11, 16.67, and 72.22% were positive for *hlyA*, *eaeA*, and *stx2*, respectively. None of the isolates carried the *stx1* gene (Olaitan et al. 2025). Elghayaty et al. (2020) revealed that the different virulence genes of *E. coli* in raw beef and some meat products in Port Said, Egypt, showed that 60, 40, and 60% of strains carried the *iss*, *iutA*, and *Trat* genes, respectively.

Out of 33 isolates obtained from beef samples, one isolate (3%) carried the *stx1* gene, 23 isolates (70%) were positive for the *stx2* gene, and nine isolates (27%) carried both *stx1* and *stx2*. Moreover, five of these isolates harbored both *stx2* and *eae* genes, with four identified as carrying the *stx2a* subtype (Egerv¨arn and Flink 2024). Despite this, the *eae* gene was absent in 25 of the isolates. Nonetheless, *eae*-negative strains have been documented to induce infection (Franz et al. 2015, Otero et al. 2017; EFSA, 2020).

Monitoring the occurrence of *E. coli* in animals and food products of animal origin is essential for maintaining public health (Ramos et al. 2020). Of particular concern is the increasing ability of organisms to develop resistance to antimicrobials among livestock (Palma et al. 2020). As a result, the effectiveness of commonly used antibiotics is diminishing, limiting treatment options and potentially leading to chronic and more severe *E. coli*-associated diseases. Therefore, antimicrobial resistance in *E. coli* poses a serious global health threat.

In this study, all 16 *E. coli* isolates tested for the *tetA* gene and the *sul1* gene showed positivity rates of 13 (81.25%) and 12 (75%,) respectively. However, all isolates tested negative for the *mrc1*, *aac(3)-IV*, and *dfrA1* genes. The RTE-derived *E. coli* co-expresses genetic resistance determinants such as ESBL, *mcr1*, and

Escherichia coli derived from ready-to-eat meats express genetic resistance factors such as ESBL, *mcr1*, and *norA*, as well as a high percentage of MDR phenotypes, which could indicate an increasing trend in antibiotic resistant *E. coli* inhabiting raw and RTE meat products. These findings, including the high prevalence and resistance of the disease, increase the risk of transmission between animals and humans and complicate treatment options (Masood *et al.* 2025).

Multidrug-resistant *E. coli* was present in raw foods (73.1%), and all five *E. coli* isolates contained blaCTX *blaCTX* and *blaSHV* genes, and *mcr1* was found in three of them, two raw and one RTEM. Two isolates of *E. coli* co-expressed *blaTEM*, *blaCTX*, and *blaSHV*, and one of them also shared *mcr1* (Masood et al. 2025).

In India, 27 isolates were verified as *E. coli*, 5 of which were ESBL positive; the most abundant genes were *blaTEM* , *blaCTX* , *blaSHV*, and *blaNDM* with incidences of 40.68, 32.20, 10.17, and 10.17%, respectively (Giri et al. 2021). All serotypes had 100% resistance to erythromycin, amoxicillin-clavulanic acid, and penicillin, and genetically, *blaTEM* and *blaSHV* were the most prevalent genes (Hemeg 2018).

Colistin resistance in *E. coli* appears to be linked to the global usage of colistin in veterinary medicine (Binsker et al. 2022). At first, chromosomal gene mutations led to colistin-resistant mechanisms, but plasmid-mediated and transmissible colistin resistance (*mcr*) led to more significant problems (Binsker et al. 2022). In an earlier Egyptian study, out of 210 *E. coli* strains (150 from raw beef and 60 from RTE beef products), eight (six strains from five raw beef and two from two RTE sausage sandwiches) were colistin-resistant and carried the *mcr*-1 gene, while five were cefotaxime-resistant and carried the *blaCTX*-M-28 gene, and three carried both *mcr*-1 and ESB (Sabala et al. 2021).

Improper food handling and preparation, such as not washing hands thoroughly or using contaminated utensils, can increase cross-contamination. Additionally, improper storage practices, such as not separating raw products and ready-to-serve items, or maintaining improper temperatures, can enable bacteria to persist (Pakdel et al. 2023).

Conclusion

This study demonstrated the presence of Shiga toxin-producing *E. coli* (STEC), a foodborne pathogen, was detected in 16% of ready-to-eat sausage samples collected from Giza and Cairo, Egypt. An elevated prevalence of resistance to ampicillin, erythromycin, and colistin (100%) was determined, followed by cefotaxime (81.25%), tobramycin (62.5%), tetracycline, and trimethoprim-sulfamethoxazole (43.75%), and ciprofloxacin (25%), while chloramphenicol and gentamicin showed the lowest resistance at 12.5%.

Regarding virulence and antibiotic resistance genes, all *E. coli* isolates were positive for *stx1*, *stx2*, and *iss* genes, with positivity rates of 31.25, 93.75, and 50%, respectively. However, all isolates were negative for *eaeA*, *astA*, and *papC* genes. In addition, the study showed positive rates for the *tetA* and *sul1* genes for all *E. coli* isolates, reaching 81.25 and 75%, respectively. However, all isolates are negative for the *mrc1*, *aac(3)-IV*, and *dfrA1* genes.

These results emphasize the importance of continuous monitoring, periodic diagnosis of STEC cases, and improved hygiene, which are essential for ensuring the safety of ready-to-eat meat products and protecting public health in Egypt.

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Author's Contribution: AAA and MMK participated in the study design. AAA performed PCR assays for genetic markers of virulence and antimicrobial resistance, project management, and reviewed the manuscript. MMK was responsible for serotyping, antimicrobial activity detection, manuscript drafting, and editing.

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