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Detection of Extended-Spectrum β-Lactamases Producing *Escherichia coli* from Beef in Mansoura, Egypt

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Abstract

The objective of the current study was to identify extended-spectrum β-lactamase-producing *Escherichia coli* (ESBLs) from beef meat in Mansoura City, Egypt. Randomly selected 150 buffalo meat samples were obtained from retail butcher shops located in Mansoura City, Egypt. Samples were inoculated into enriched broth media and then directly streaked on *E. coli* selective agar for selective isolation of *E. coli*. A biochemical examination was performed for the preliminary identification of *E. coli*. Suspected *E. coli* isolates were then confirmed by PCR targeting 16S rRNA. Confirmed *E. coli* isolates were tested for their susceptibility to antimicrobials by using Kirby–Bauer disc diffusion method. Additionally. *E. coli* were tested for the presence of β-lactamases (bla_{CTX}, and bla_{SHV} and bla_{TEM}) using PCR. *E. coli* was isolated from 37 out of 150 tested samples with a total prevalence of 24.66 %. *E. coli* isolates revealed a high resistant against cefotaxime and ceftazidime, and moderate resistance against amoxy clavulanic acid, while a high sensitivity of *E. coli* was displayed against meropenem and imipenem. Moreover, the tested *E. coli* (*n.*=37) harbored ESBLs genes with percentages of 40.54% for both bla_{CTX}, and bla_{SHV} and 35.13% for bla_{TEM}. *E. coli* produces extended-spectrum β -lactams that were isolated from meat and contained a substantial level of antibiotic resistance genes. To increase food processing quality and provide safe food for consumers, it is crucial to build food traceability and monitoring systems for meat and meat products.

KEYWORDS
E. coli, Beef, ESBL, Antimicrobials susceptibility

INTRODUCTION

Escherichia coli is one of the principal bacteria responsible for human foodborne illnesses connected to meat. Bacteria including multidrug-resistant (MDR) *E. coli*, have been detected in high proportions in meat, especially beef, intended for human consumption (Gregova *et al.* 2020). Extended-spectrum β -lactamases (ESBLs), which hydrolyze and lead to resistance to different types of β-lactam antibiotics are produced by some strains of MDR bacteria. Broad-spectrum cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime, are among them. β-lactams most likely contain the most chemical alterations of any antibiotic family. A wider range of enzyme activity has been observed as a result of gene diversification in the β-lactams family. Over 300 ESBL enzymes are known; TEM, SHV, and CTX are the most prevalent, with CTX having the greatest global spread. Although commensal ESBL strains can remain with their host without harm, resistance development can take place in these strains, with the potential to spread to other commensal or pathogenic bacteria (Martínez-Vázquez *et al*., 2022).

Previous studies have shown that animal *E. coli* strains are responsible for antibiotic-resistant *E. coli* infections in humans (Johnson *et al*., 2009). Contamination of meat during slaughter, preparation, and processing may result in the transfer of antibiotic-resistant *E. coli* to the meat. The overuse of antimicrobials in the animal agriculture industry encourages the proliferation of multidrug-resistant bacteria in animals used for food production, which could result in the spread of these bacteria in foods derived from these animals, such as meat and its byproducts (Cheng *et al*., 2019). In both human and animal medicine, given the limited number of effective treatments for multidrug-resistant *Enterobacteriaceae* infections.

Resistance to third generation cephalosporins in bacterial pathogens is a serious problem (Greko, 2009). The synthesis of beta-lactamases (ESBL), a type of resistance mechanism, is the most prevalent one in the third generation of cephalosporin-resistant *Enterobacteriaceae*. Because the enzymes are typically encoded on plasmids, conjugation/mobilization is a major factor in the establishment and spread of ESBL-producing *Enterobacteriaceae* (Bonnet, 2004)

β-lactamase synthesis is the most prevalent mechanism of drug resistance, notably in Gram-negative bacteria, and it is a growing issue regarding resistance to β-lactam antibiotics. As a result of ongoing mutation, β-lactamases have remarkable diversity. Extended-spectrum β-lactamases (ESBLs) are particularly dangerous among them. The majority of ESBL reports come from the *Enterobacteriaceae* family. TEM-type and SHV-type ESBLs dominated the ESBL market in the 1990s. In the last ten years, quick and widespread dissemination of ESBLs of the CTX type has been reported (Mora-Ochomogo *et al*., 2021). Therefore, this study was conducted to investigate the prevalence of extended-spectrum β -lactamases -producing *E. coli* isolated from marketed meat in Mansoura city.

MATERIALS AND METHODS

Ethical approval

This study was ethically approved by Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Code No: M/69)

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Study area

This research was carried out at Mansoura, Egypt, the capital of the Dakahlia governorate. Mansoura is a city in Egypt's northwestern region. Dakahlia governorate is considered one of Egypt's most populous governorates, has a significant agriculture industry, and a high population of ruminants mainly buffaloes and cattle which used as a primary source for meat production.

Study design and sampling strategy

The study was conducted on 150 raw meat samples that were collected from retail shops and supermarkets located at Mansoura city, Dakahlia governorate, Egypt. From January to March 2022 All samples were transported in sterile containers to the Bacteriology laboratory, Faculty of Veterinary Medicine, University of Mansoura, Egypt, and examined within 24 h of collection.

Isolation and identification of E. coli

About 25 g of each beef sample were inoculated in 225mL of Tryptone Soy Broth (TSB, Oxoid, USA) and incubated at 37°C for 24 h. Following that, a loopful was streaked on Eosin methylene blue (EMB) agar plates (Oxoid) and incubated for 24 h at 37°C. The typical characteristic colonies of *E. coli* (greenish metallic sheen) were harvested and purified on Trypticase Soy Agar (TSA) for further biochemical characterization using different sets of biochemical tests which included IMViC, catalase, oxidase, and sugar fermentation according to Collee *et al.* (1996).

Molecular characterization of E. coli.

DNA extraction

Three to five bacterial colonies were collected from TSA agar plates, homogenized in 200 µL deionized water, boiled for 15 min (dry bath incubator), and centrifuged for 3 min at 10,000 rpm. The supernatant was transferred to a sterile Eppendorf tube and was used as a DNA template. DNA samples were stored at -20°C until used (Ramadan *et al*., 2016).

Molecular characterization of *E. coli* isolates

PCR was performed in a 96-well 2720 thermocycler (Applied Biosystems, US). All the PCR amplifications for detecting the different genes in this study were performed in 25 µL reaction volume containing 12.5 µL of 2X TOPsimple™ PCR DyeMIX-HOT (P510H) (enzynomics) PCR Master Mix, 5.5 µl of sterile water, 1µL of both forward and reverse primers, and 5 µL of the DNA template. All suspected isolates were screened for the species-specific gene16S rRNA using PCR cycling conditions described by Amit-Romach *et al.* (2004). The PCR products were visualized by electrophoresis on 1.5% agarose gels stained by ethidium bromide on UV transillumination. Samples showed an amplicons size of 585 bp is considered positive.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of *E. coli* was tested using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar plates (Oxoid, UK), as per the Clinical and Laboratory Standard Institute (CLSI, 2019) recommendations. The antibiotics used (Bioanalyse, Turkey) were amoxy clavulanic acid (AMC, 25 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), meropenem (MEM, 10 µg), and imipenem (IPM, 10 µg). The results were interpreted according to the CLSI (2019) recommendations after 24 h of incubation at 37°C.

Phenotypic screening and confirmation of ESBL

The tested *E. coli* strains were streaked on Mueller–Hinton agar (Oxoid, UK). The amoxy clavulanic AMC (20/10 µg) disc was placed in the center of the plate, surrounded by discs of ceftazidime CAZ (30 µg), and cefotaxime CTX (30 µg). All the discs were placed 20 mm from each other. After 24 h incubation at 37°C, enhancement of the zone of inhibition towards the AMC disc indicated a potential ESBL-positive strain.

Genotypic screening and detection of ESBL

Polymerase chain reaction (PCR) was performed to investigate the presence of ESBLs-encoding genes, including bla_{TEM}, bla_{SHV}, and bla_{CTX} using PCR. The oligonucleotide primers are illustrated in Table 1 as previously reported by Colom *et al*., (2003). The reaction was performed as mentioned above using the following thermal condition: one cycle at 94°C for 5 min, 30 cycles at 55°C for 45 sec and at 72°C for 1 min, and finally, extension cycle at 72°C for 10 min (Table 2). PCR products were visualized in agarose gel electrophoresis, using 1.5% agarose stained by ethidium bromide, and photographed by the gel imaging system.

RESULTS

E. coli prevalence in commercially available beef cuts

Of 150 meat cut samples investigated in this study, 37 isolates were recovered with an overall prevalence of 24.66%. The growing colonies of *E. coli* had a characteristic metallic green sheen on EMB agar. Biochemically, all recovered strains were positive to catalase, methyl red, indole, and sugar fermentation tests, while they reacted negatively to Voges–Proskauer, citrate utilization, oxidase and H_2S production tests. After Gram staining, the bacteria were Gram-negative, rod-shaped bacilli, motile, and non-spore-forming.

Table 1. Oligonucleotide primers that were used in this study.

Genes	Primer Sequence (5′–3′)	Amplicons (bp)	References
16S rRNA	F- GACCTCGGTTTAGTTCACAGA R- CACACGCTGACGCTGACCA	585	Amit-Romach *et al.* (2004).
bla_{TEM}	F- ATCAGCAATAAACCAGC R- CCCCGAAGAACGTTTTC	516	Colom *et al.* (2003)
bla_{SHV}	F- AGGATTGACTGCCTTTTTG R- ATTTGCTGATTTCGCTCG	392	Colom *et al*. (2003)
bla_{CTX}	F-ATG TGC AGY ACC AGT AAR GT R-TGG GTR AAR TAR GTS ACC AGA	593	Archambault *et al.* (2006)

Table 2. Cyclic conditions used in PCR reactions.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
16S rRNA	94˚C, 5 min.	94˚C, 30 sec.	60˚C, 1 min.	62˚C, 2 min	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, 45 sec	35	72˚C, 10 min
bla_{CTX}	94˚C, 3 min.	94˚C, 45sec	45˚C, 45 sec	72˚C, 45 sec	30	72˚C, 10 min.

Table 3. Antimicrobial susceptibility testing results.

Antibiotic	Disk code	Resistant	%	Intermediate	%	Sensitive	%
Amoxy clavulanic acid	AMC	6	16.2	8	21.6	23	62.1
Ceftazidime	CAZ	12	32.4	16	43.2	9	24.3
Cefotaxime	CTX	25	67.5	8	21.6	4	10.8
Meropenem	MEM	0	0	4	10.8	33	89.2
Imipenem	IPM	5	13.2	8	21.6	24	64.9

Antimicrobial susceptibility testing and ESBL test for E. coli isolated strains

Antimicrobial susceptibility testing was conducted on all retrieved *E. coli* isolates. *E. coli* isolates showed various resistance patterns against the employed antibiotics. The resistance of bacterial isolates was high against cefotaxime (67.5%; 25/37) and ceftazidime (32.4%; 12/37) and moderate to amoxy clavulanic acid (16.2%; 6/37) while a high sensitivity to meropenem (100%; 0/37) 0and imipenem (86.4%; 5/37) was displayed as shown in Table 3.

The Double Disc Synergy Test was performed on all the retrieved strains for detection of ESBL production, the enhancement of the zone of inhibition towards the AMC disc indicated a potential ESBL-positive strain. Among the tested *E. coli* strains, 15 isolates showed positive results in ESBL test (Figure 1).

Figure 1. Double disc synergy test showing an enhancement of zone towards amoxicillin-clavulanic acid.

Moreover, the tested *E. coli* (*n.*=37) was tested for the presence of ESBLs encoding genes. The targeted genes were successfully amplified at 516 bp, 392 bp and 593 bp for bla_{TEM}, bla_{SHV} and bla_{CTX} respectively (Figures 2, 3, 4). ESBLs genes were detected with percentages of 40.54% for both bla_{CTX}, and bla_{SHV} and 35.13% for bla_{TEM} from the overall tested isolates (Table 4).

Table 4. Prevalence of ESBLs encoding genes in *E. coli* isolates.

Gene	Positive	%	Negative	%
bla_{CTX}	15	40.54%	22	59.46%
bla_{SHV}	15	40.54%	22	59.46%
bla_{TEM}	13	35.13%	24	64.86%

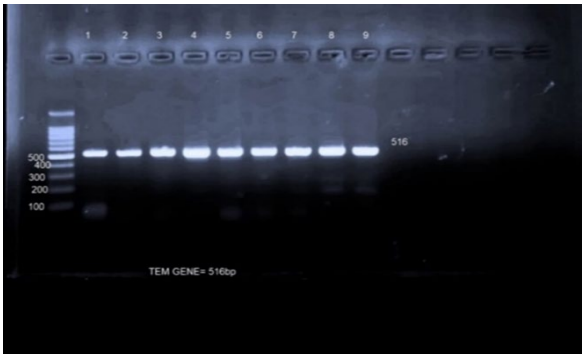

Figure 2. Agarose gel electrophoresis showing amplification of bla_{TEM} gene (516 bp).

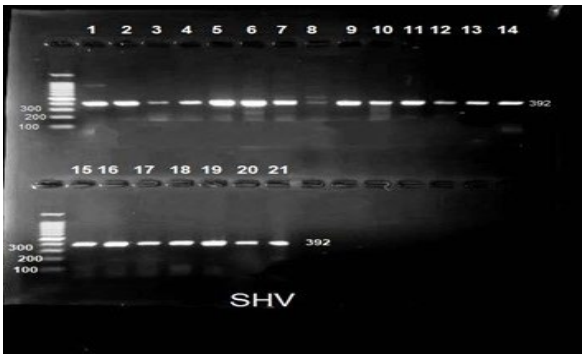

Figure 3. Agarose gel electrophoresis showing amplification of bla_{SHV} gene (392 bp).

Figure 4. Agarose gel electrophoresis showing amplification of bla_{CTX} gene (593 bp).

DISCUSSION

One of the most rapidly spreading resistance issues globally is caused by *Enterobacteriaceae*, which produce extended-spectrum

-lactamases and carbapenems. Livestock may play a significant role in the spread of ESBL and carbapenem-producing bacteria throughout the population. Animals used for food production in Egypt have not been completely evaluated, and the possibility of ESBL or producing *E. coli* and their encoding genes contaminating red meat is unknown (Abdallah *et al*., 2022). According to recent research, ESBL-producing *E. coli* may also cause infections that are acquired in the community. The effectiveness of penicillin and cephalosporins for treating infections by the spread of ESBL-producing and *E. coli* outside of the hospital setting (Kawamura *et al*., 2017). In Algeria and Korea, ESBL-producing *E. coli* has reportedly been found in raw meat from livestock, ready-to-eat sandwiches, and vegetables. (Kawamura *et al*., 2017). However, ESBL producers have not been found in fruits and vegetables in Switzerland or the UK (Kawamura *et al*., 2017). In our study, we aimed to detect the prevalence of the three genes of the ESBL group in meat marketed in Mansoura city to establish a recognizable record of the occurrence of those genes to estimate the risk of repeated exposure to bacterial strains carrying the resistance genes. The result of this study showed that the prevalence of *E. coli* strains isolated from marketed meat was 24.66%. On the other hand, chicken meat in Egypt seemed to be more infected than buffalo meat (Abdallah *et al*., 2022). In our study, the frequency of ESBL-producing *E. coli* was higher than that found in sheep meat in Switzerland (8.6%) and Portugal (5.5%), but it was lower than that found in Iran (60%) and Tunisia (63.8%) in chicken meat. Twenty-three percent was discovered among imported chicken meat in Gabon and 27.5% was discovered in ground beef samples from Algeria according to Abdallah *et al*. (2022). Our findings are consistent with an earlier study from Egypt, where bla_{CTX}, bla_{TEM}, and bla_{SHV} were discovered in *E. coli* isolated from meat and dairy farms (Braun *et al*. 2016), our data showed that bla_{CTX}- and bla_{SHV} were the most frequent ESBL-types in our *E. coli* collection. The bla_{CTX} was screened which identify as similar to species- specific beta lactmases from *Kluyvera* spp (Bonnet *et al*., 2004). bla_{CTX}-M is one of the most prevalent ESBL genes, and its frequency has been increasing in Western and Central Europe for several years (Eskandari-Nasab *et al*., 2018). Our result reveled bla_{CTX}-gene in 15 isolates of 37 *E. coli* isolates (40%) that lower than Kingsley and Verghese, (2008) who revealed that CTX- gene found in 45% of *Klebsiella* spp , *E. coli* and *Enterobacter* spp taken from patients in India. Moreover, Lin *et al*., (2010) reported that CTX-M-14type was the more frequent ESBLs between *E. coli* taken from regional hospitals in Taiwan with prevalence (53.6%) and Hammad and Shimamoto (2011) who recorded that CTX- gene found in 8.6% of animals in Japan, in 2009, that unlike the higher rate of CTX- gene isolated from humans. The second gene to be screened was $_{SHV}$, which can be characterized as one of the ESBLs produced by chromosomal genes in isolates of *K. pneumonaie* distributed by mobile elements as plasmids between microbial communities, particularly the *Enterobacteriaceae* family (Paterson and Bonomo, 2005). It is highly significant to screen ESBLs because it is one of the most important reasons for treatment failure (Netzel *et al*., 2007). Result of SHV-was 40% which was lower than Arabi *et al*., (2015) who reported 53.2% of ESBL–producing isolates had bla_{SHV}. Also, Tasli and Bahar, (2005) who revealed bla_{SHV} in 74.3%, Cheong *et al*., (2014) determined that 9% of isolates were positive for bla_{SHV} and Dallal *et al*., (2013) recorded that 6.1% isolates contain bla_{SHV} .Regarding bla_{TEM} gene it was lower than that of Park *et al*., (2005) who detected bla_{TEM} in (46.3%) taken from *E. cloacae*, *C. freundii*, *S. marcescens*. Also, Hammad and Shimamoto (2011) who identify bla_{TEM} in 26% of *E. coli* and K. pneumoniae isolates (23) from milk samples and Ogbolu *et al*., (2013) who reported that 47 from 63 *K. pneumonaie* isolated in Nigeria from clinical specimens had TEM-1(74.6%) and also reported that *E. coli* had 24 (85.7%) of TEM-1.Therefore, this study is considered one of the important studies in the Delta region, which highlights the importance of establishing a system to reduce the impact of transmission of antibiotic resistance through various food sources, especially meat.

CONCLUSION

E. coli produces extended-spectrum β -lactams that were isolated from meat and contained a substantial level of antibiotic resistance genes. To increase food processing quality and provide safe food for consumers, it is crucial to build food traceability and monitoring systems for meat and meat products.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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