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Prevalence of Multidrug Resistant Shiga Toxin-Producing *Escherichia coli* in Cattle Meat and Its Contact Surfaces

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HIGHLIGHTS

- Prevalence of *Escherichia coli* in the chuck, round, masseter muscles, cutting-boards, walls, and floors were 20, 10, 30, 50, 40, and 60%, respectively.
- The isolated *E. coli* serovars showed a multidrug resistance profile.
- Cutting-boards, walls, and floors are possible sources for contamination of meat with *E. coli*.

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Acronyms and abbreviations
STEC=Shiga Toxin-producing *E. coli*
PCR=Polymerase Chain Reaction
MPN=Most Probable Number

ABSTRACT

Background: Shiga toxin-producing *Escherichia coli* (STEC) are group of *E. coli* causing bloody diarrhea. The goal of this survey was to determine the prevalence of multidrug resistant shiga toxin-producing *E. coli* in cattle meat and its contact surfaces.

Methods: Swab samples (n=120) were randomly collected from meat and contact surface of butchery shops in Sharkia province, Egypt. Prevalence of *E. coli* was examined using culture, biochemical, and serological methods. Identification of shiga toxin-encoding genes (*stx1* and *stx2*) in the *E. coli* serotypes was done using multiplex polymerase chain reaction. Screening of multidrug resistance profile was done using the disk-diffusion method. Data were analyzed using JMP statistical package, SAS Institute Inc., Cary, NC.

Results: The prevalence rates of *E. coli* in the chuck, round, masseter muscles, cutting-boards, walls, and floors were 20, 10, 30, 50, 40, and 60%, respectively. Among the isolates, *E. coli* O111:H4 and *E. coli* O26:H11 harbored the two mentioned genes. *E. coli* O86 and *E. coli* O114:H21 harbored only *stx1*; while *E. coli* O55:H7 encoded only *stx2*. Just *E. coli* O124 had no express of *stx1* and *stx2*. The isolated *E. coli* serovars showed a multidrug resistance profile.

Conclusion: Considering the results of this study, strict hygienic procedures should be followed to avoid or reduce carcass cross-contamination. In addition, proper handling and efficient cooking of meat are highly recommended by consumers to reduce the risk of human exposure to STEC.

Introduction

Escherichia coli is dominant intestinal flora that live commensally in the intestinal tracts of humans and animals. Some *E. coli* strains are pathogenic and either cause localized disease that limited to the intestinal tract

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with diarrhea as a major symptom or extra intestinal infection (Xia et al., 2010). Such intestinal pathotypes of *E. coli* are called diarrheagenic *E. coli* and can be transmitted via ingestion of contaminated food and water. Pathotypes of *E. coli* are classified into six categories namely enterohemorrhagic *E. coli*, also called shiga toxin-producing *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli*, and diffusely adherent *E. coli* (Darwish et al., 2015; Xia et al., 2010).

Shiga Toxin-producing *E. coli* (STEC), also called verocytotoxic *E. coli*, causes bloody diarrhea as a major symptom. *E. coli* O157:H7 is the major STEC serotype responsible for *E. coli* outbreaks in North America. *E. coli* O104:H4 was the responsible for *E. coli* outbreak in Europe during 2011 (Frank et al., 2011). Other STEC serotypes include O26, O111, and O103. The major symptoms of STEC infections are abdominal cramps, bloody diarrhea, vomiting, and slight fever. About 5-10% of the infected people may develop hemolytic uremic syndrome, a life threatening kidney disease with kidney failure-like symptoms (CDC, 2018).

Cattle meat is a main source of essential amino acids, minerals, fatty acids, and also vitamins. However, such important meat source was linked to several STEC outbreaks worldwide. For instances, ground beef was associated with an outbreak of STEC O157:H7 in USA in 2014, with 12 infected persons (CDC, 2014). Furthermore, ground beef was recently linked to an outbreak of STEC O26 in USA with 18 infected people, 6 hospitalized people, and one died person in Florida (CDC, 2018).

Cattle meat could be contaminated with different pathogenic organisms, including *E. coli* due to direct contact with contaminated surfaces or equipment such as butcher hands, knives, cutting boards, walls, floors, air, and water (Darwish et al., 2016; McEvoy et al., 2003). This cross-contamination takes place at different stages of processing starting from animal slaughtering, skinning, evisceration, de-boning, carcass transportation, and also distribution (Borch and Arinder, 2002).

The abuse of antibiotics in livestock production for disease prevention and control had resulted in development of antimicrobial resistant bacterial strains, which have several critical effects on public health (Darwish et al., 2013). Also, the uncontrolled usage of antimicrobials in animal farms may result in antimicrobial residues in the food products of these animals, which may have several toxicological implications. For instances, high intake of the residues of tetracyclines and quinolones may lead to adverse effects, including nephropathy, anaphylaxis, and teratogenesis (Kools et al., 2008).

In sight of the previous factors, this study was undertaken to investigate the prevalence of STEC in the cattle chuck, round, and masseter muscle retailed in Sharkia Governorate, Egypt. To investigate the possible sources of carcass contamination with STEC, the prevalence rates of STEC were investigated in the cutting-boards, walls, and floors of the same butchery shops. *E. coli* strains were serologically identified. Identification of shiga toxin-encoding genes, including *stx1* and *stx2* was also examined using Polymerase Chain Reaction (PCR). Meanwhile, antimicrobial resistance profiles of the identified serotypes were evaluated by disk diffusion assay.

Materials and methods

Collection of samples

Swab samples (n=120) were collected randomly and equally (n=20 of each sample type) from chuck, round, master muscles, cutting-boards, walls, and floors of different butchery shops in Sharkia province, Egypt. Each swab sample represents a space area of 1x1 cm^2 from the surface of the meat or its contact surfaces (APHA, 2001). Sample collection was done from April 2016 to February 2017.

Microbiological examinations

Sampling of the surface of each sample was conducted using sterile gauze swabs moistened in a sterile 0.9% saline solution followed by sampling by dry swabs. Each of two used swabs was placed into a sterile test tube containing 10 ml of a sterile 0.9% saline solution, shaken vigorously and considered as 10^{-1}; then decimal up to six dilutions were done (APHA, 2001).

Determination of Most Probable Number (MPN) of coliforms

One ml of each dilution was inoculated separately into three test tubes containing MacConkey broth (Merck, Darmstadt, Germany) with inverted Durham's tubes. The inoculated tubes were incubated at 37 ºC for 24 h. Positive tubes showing acid (yellow color) and gas production in inverted Durham's tubes were recorded. The MPN of coliforms was calculated according to the recommended tables (APHA, 2001).

Determination of MPN of E. coli

One ml of each positive dilution was moved separately to another three test tubes containing EC broth (Merck, Darmstadt, Germany) with inverted Durham's tubes, then incubated at 44.5 ºC for 24 h. Positive tubes showed gas production with turbidity of the broth. MPN of *E. coli* was calculated according to the recommended tables same as MPN of coliforms (APHA, 2001).

Isolation of E. coli

One loopful of the positive tubes in MPN of *E. coli* was streaked onto MacConkey agar plates (Difco, Detroit, MI, USA), and then incubated at 37 °C for 24 h in aerobic conditions. The lactose fermenting colonies were reinoculated to Eosin Methylene Blue agar plates (Difco, Detroit, MI, USA) and incubated at 35 °C for 24 h. Metallic sheen-producing colonies were transferred to Nutrient agar slants, incubated at 37 °C for 24 h and then stored at 4 °C for further analysis. Identification of isolates was done based on Gram staining and biochemical tests, including catalase, oxidase, indol production, methyl red, Voges-Proskauer test, citrate utilization, nitrate reduction, urease, H_2S production, gelatin liquefaction, and Eijkman test.

Serodiagnosis of E. coli

The confirmed *E. coli* strains were serologically identified by rapid diagnostic *E. coli* antisera sets (Difco, Detroit, MI, USA) for diagnosis of the Enteropathogenic types (Kok et al., 1996).

DNA preparation and PCR amplification of shiga toxin-encoding genes

DNA extraction was done using QIAamp DNA kit (Cat No. 51304, Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was evaluated by Nanodrop (ND-1000, Nanodrop Technologies, Wilmington, DE, USA). The primers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Primer sequences for *stx1* were sense 5′-ACACTGGATGATCTCAGTGG-′3 and antisense 5′-CTGAATCCCCCTCCATTATG-′3, and the ones for *stx2* were sense 5′-CCATGACAACGGACAGCAGTT-′3 and antisense 5′-CCTGTCAACTGAGCAGCACTTTG-′3 (Gannon et al., 1992). A multiplex PCR amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR reaction (20 µl) consisted of nucleic acid template (30 ng), 0.5 µM concentrations of each primer, 0.25 µM dNTP mixtures, 1X Ex Taq reaction buffer, and 1 U EX Taq DNA polymerase (TaKaRa, Japan). Amplification conditions consisted of an initial 95 °C denaturation step for 3 min followed by 35 cycles of 95 °C for 20 s, 58 °C annealing for 40 s, and 72 °C for 90 s. The final cycle was followed by 72 °C final extension for 7 min. *E. coli* O157:H7 Sakai (positive for *stx1* and *stx2*) was used as a positive reference strain and *E. coli* K12DH5α was used as a negative control. Amplified DNA fragments were studied by 2% agarose gel electrophoresis (Applichem, GmbH, Germany) in 1X TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, GmbH, Germany) was used to determine the fragment sizes.

Antibiotic susceptibility testing

Antibiotic susceptibility test was performed using the disk diffusion method. Briefly, antibiotic disks were placed on nutrient agar plates after inoculation and spreading of bacterial suspension. Diameters of the inhibition zones were measured after incubation time. The selected antibiotics were based on EFSA recommendations in the antimicrobial resistance monitoring studies (EFSA, 2012). The antimicrobials were ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), oxacillin (1 µg), oxytetracycline (30 µg), penicillin (10 IU), and trimethoprim/sulfamethoxazole (25 µg).

Statistical analysis

All MPN values are expressed as means±SD (MPN/cm^2), and all measurements were carried out in duplicates. Statistical significance was evaluated using the Tukey-Kramer HSD test (JMP statistical package; SAS Institute Inc., Cary, NC). In all analyses, $p<0.05$ was taken to indicate statistical significance.

Results

The present study indicated that the average values of the MPN of coliforms (MPN/cm^2) in the chuck, round, and masseter muscles were 2.75±0.22, 2.55±0.32, and 3.55±0.25, respectively; these values were 5.40±0.45, 5.48±0.55, and 6.50±0.14, respectively, in the swab samples collected from the cutting-boards, walls, and floors of the butcher shops (Figure 1-A). In parallel, the mean±SD values of MPN of *E. coli* (MPN/cm^2) in the chuck, round, and masseter muscles were 2.10±0.11, 2.20±0.22, and 3.00±0.24, respectively. Such values in the meat contact surfaces, including cutting-boards, walls, and floors of the butcher shops were 3.60±0.22, 4.20±0.32, and 4.80±0.16, respectively (Figure 1-B).

The prevalence rates of *E. coli* in the chuck, round, masseter muscles, cutting-boards, walls, and floors were 20, 10, 30, 50, 40, and 60%, respectively. Six pathovars of *E. coli* were serologically identified, including *E. coli* O55:H7, *E. coli* O86, *E. coli* O111:H4, *E. coli* O114:H21, *E. coli* O124, and *E. coli* O26:H11 at variable percentages (Figure 2). The identified pathovars were screened for harboring shiga toxin-encoding genes (*stx1* and *stx2*). The obtained results indicated that *E. coli* O111:H4 and *E. coli* O26:H11 harbored the two mentioned genes. *E. coli* O86 and *E. coli* O114:H21 harbored

only *stx1*; while *E. coli* O55:H7 encoded only *stx2*. Just *E. coli* O124 had none of the mentioned genes, including *stx1* and *stx2*.

The current investigation was extended to examine the antimicrobial resistance profile among the isolated *E. coli*. The presented results in Table 1 showed that 42 (100%) of the isolated *E. coli* strains were resistant to ampicillin, nalidixic acid, and penicillin; while 36 (85.68%), 34 (80.92%), 30 (71.40%), 28 (66.64%) and 18 (42.84%) of the isolates were respectively resistant to oxacillin, trimethoprim/sulfamethoxazole, oxytetracycline, cephalothin, and ciprofloxacin. On the other hand, 100% of the identified *E. coli* stains were susceptible to kanamycin, while 64.3-80.96% of isolates were susceptible to gentamicin, chloramphenicol, erythromycin, enrofloxacin, and neomycin. The identified *E. coli* serovars showed variable degrees of resistance to the most commonly used antimicrobials in Egypt (Table 1).

Discussion

Microbial contamination of meat with food-poisoning microorganisms such as *E. coli* usually starts at slaughterhouses and/or butcher shops. Consumption of such contaminated meats may increase the risk of exposure to food poisoning and result in several implications on both public health safety and economic losses (CDC, 2013). One major task for both meat and environmental hygiene is to ensure safety of the meat, meat handlers, and also consumers against food-borne pathogens. Cross-contamination of meat from meat-contact surfaces and surroundings such as cutting-boards, walls, and floors as a major cause of contamination of meat with coliforms and *E. coli* had received little attention in Egypt. Furthermore, MPN of coliforms is considered as an ideal indicator for the hygienic status of meat and its surroundings. In addition, MPN of *E. coli* provides a clear image about the sanitary status of the meat and its contact surfaces (ICMSF, 1996). The achieved results indicated that masseter muscle had both the highest MPN of coliforms and of *E. coli*. This result might be explained as this meat part is considered as an offal part near the site of slaughter and receives little attention during meat cutting and preparation. In general, MPN of coliforms and *E. coli* were high in all examined meat-contact surfaces, including cutting-boards, floors, and walls when compared with the muscle samples. Among these contact surfaces, floors had the highest MPN of coliforms and *E. coli*. This result declares inadequate hygienic measures adopted during slaughtering, evisceration, dressing, or preparation of meat. This result was comparable to that recorded in Australian sheep meat (Vanderlinde et al., 1999). In Egypt, the recorded results go in agreement with Algabry et al. (2012), who reported high total coliform counts in cattle carcasses and their contact surfaces in butcher shops at Alexandria province.

Table 1: Percentage of antimicrobial resistant isolates among identified *Escherichia coli* serotypes

Antibiotic type	*E. coli* (Total=42)		*E. coli* O55:H7 (Total=8)		*E. coli* O86 (Total=7)		*E. coli* O111:H4 (Total=8)		*E. coli* O114:H21 (Total=4)		*E. coli* O124 (Total=6)		*E. coli* O26:H11 (Total=9)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Ampicillin	42	100.0	8	100.0	7	100.0	8	100.0	4	100.0	6	100.0	9	100.0
Cephalothin	28	66.6	6	75.0	2	28.6	8	100.0	1	25.0	5	83.3	6	66.7
Chloramphenicol	10	23.8	3	37.5	0	0.0	3	37.5	0	0.0	2	33.3	2	22.2
Ciprofloxacin	18	42.8	5	62.5	1	14.3	4	50.0	1	25.0	2	33.3	5	55.6
Enrofloxacin	15	35.7	4	50.0	1	14.3	5	62.5	0	0.0	2	33.3	3	33.3
Erythromycin	12	28.6	4	50.0	0	0.0	3	37.5	0	0.0	2	33.3	3	33.3
Gentamicin	8	19.0	2	25.0	0	0.0	3	37.5	0	0.0	1	16.7	2	22.2
Kanamycin	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Nalidixic acid	42	100.0	8	100.0	7	100.0	8	100.0	4	100.0	6	100.0	9	100
Neomycin	15	35.7	4	50.0	2	28.6	5	62.5	1	25.0	1	16.7	2	22.2
Oxacillin	36	85.7	8	100.0	4	57.1	8	100.0	3	75.0	5	83.3	8	88.9
Oxytetracycline	30	71.4	7	87.5	2	28.6	8	100.0	2	50.0	4	66.6	7	77.8
Penicillin	42	100.0	8	100.0	7	100.0	8	100.0	4	100.0	6	100.0	9	100.0
Trimethoprim/ Sulfamethoxazole	34	80.9	8	100.0	5	71.4	8	100.0	2	50.0	4	66.6	7	77.8

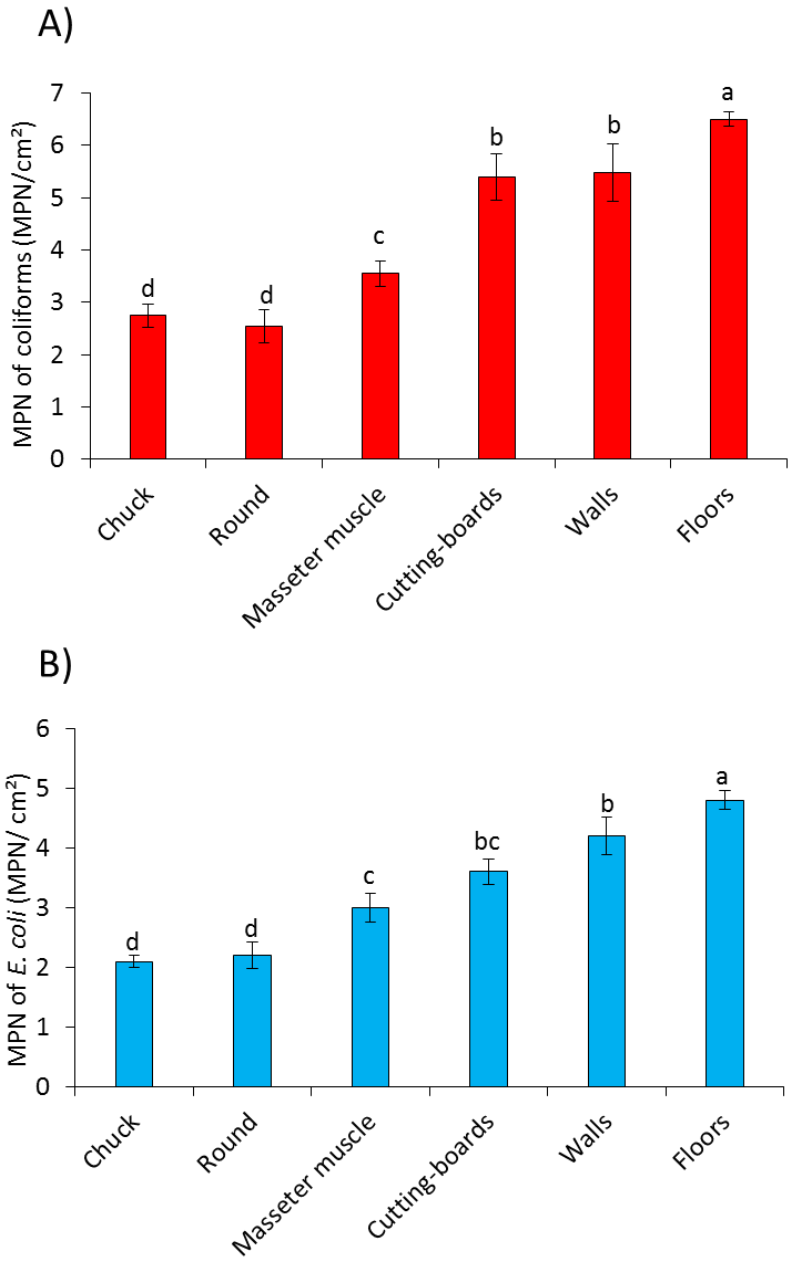

Figure 1: Most Probable Number (MPN) of coliforms and *Escherichia coli* in swabs from cattle meat and its contact surfaces. A) MPN of coliforms, B) MPN of *E. coli* in the cattle muscle samples and their contact surfaces, values represent means±SD (MPN/cm^2). Columns varying different letters are significantly different at $p<0.05$ (n=20)

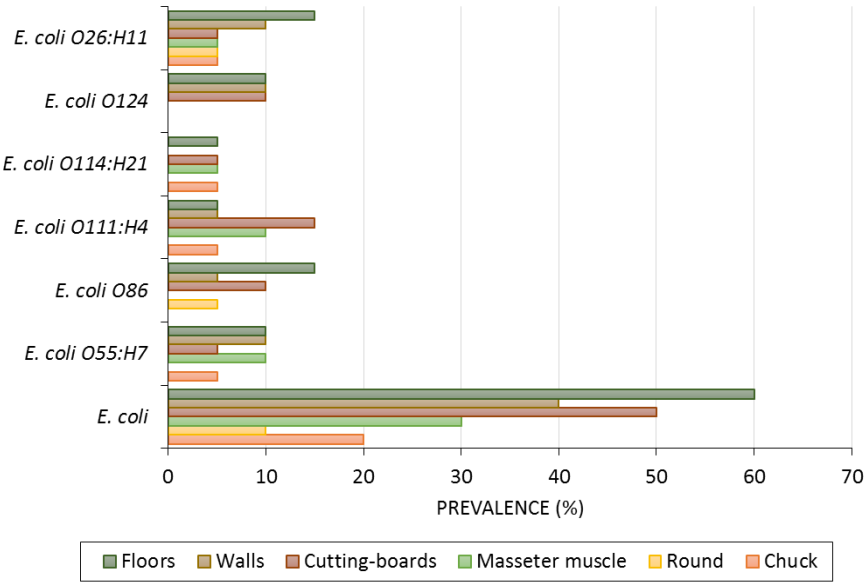

Figure 2: Prevalence (%) of different *Escherichia coli* serotypes identified in cattle meat and its contact surfaces

We further investigated the prevalence rates of *E. coli* in the examined samples, similar to MPN of *E. coli* results, swabs smeared from the floors had the highest prevalence rate of *E. coli* (60%), followed by the cutting-boards (50%), walls (40%), masseter muscles (30%), chuck (20%), and round (10%). Six serovars of *E. coli* were identified belong to EHEC (*E. coli* O111:H4 and *E. coli* O26:H11), EIEC (*E. coli* O124), and EPEC (*E. coli* O55:H7, *E. coli* O86, and *E. coli* O114:H21). The most frequently isolated serotypes were *E. coli* O26:H11 and *E. coli* O55:H7. Similarly, ETEC and EPEC serovars were isolated from imported meat, poultry, and game meat worldwide, including Malaysia (Abuelhassan et al., 2016), Germany (Mateus-Vargas et al., 2017), India (Hussain et al., 2017), Ghana (Eibach et al., 2018), and Peru (Ruiz-Roldán et al., 2018).

E. coli might also be classified into STEC (diarrheagenic *E. coli*) or non-shigatoxigenic strains (non-diarrheagenic *E. coli*) based on their abilities to produce the enterotoxin. Shiga toxin is coded by two genes namely *stx1* and *stx2* (Trabulsi et al., 2002). Therefore, the present study was extended to investigate the identification of these two genes in the identified serotypes. Interestingly, the two EHEC strains (*E. coli* O111:H4 and *E. coli* O26:H11) harbored two mentioned genes. While, *E. coli* O55:H7, *E. coli* O86, and *E. coli* O114:H21 harbored one of these genes. *E. coli* O124 had none of the studied genes. In agreement with these results, *E. coli* O55:H7 was previously isolated from an infant with diarrhea in Germany (Zhou et al., 2010). In addition, Gao et al. (2018) had reported that the major seven STEC serovars in ready-to-eat meats, fruits, and vegetables are *E. coli* O157:H7, O26, O121, O145, O45, O103, and O111. The spread of such STEC pathovars to meat might be through direct contamination through rupture of the gastrointestinal tract of the cattle during evisceration and preparation of the carcass or through cross-contamination with the fecal matter-contaminated carcass contact-surfaces. In agreement with such speculation, Mellor et al. (2016) isolated *E. coli* O157 and six non-O157 STEC serotypes, including O26, O45, O103, O111, O121, as well as O145 from some Australian beef cattle feces.

Antimicrobials are routinely used during livestock production cycle for the prevention and control of infectious diseases. However, the abuse of such antimicrobials may lead to development of antimicrobial resistant bacterial strains especially among the common inhabitant of the animal gastrointestinal tract such as *E. coli*. Therefore, the current study was extended to investigate the antimicrobial resistance profile among the identified *E. coli* serotypes. All *E. coli* isolates had 100% resistance to ampicillin, penicillin, and nalidixic acid. In addition, all *E. coli* serovars had resistance to more than one tested antibiotic showing a multidrug resistance tendency. Among the identified serotypes, *E. coli* O111:H4 had the

highest resistance profile as 100% resistance to seven tested antimicrobials, including ampicillin, cephalothin, nalidixic acid, oxacillin, oxytetracycline, penicillin, and trimethoprim/sulfamethoxazole. *E. coli* O55:H7 showed complete resistance to five antimicrobials including, ampicillin, nalidixic acid, oxacillin, penicillin, and trimethoprim/sulfamethoxazole. All identified serotypes were just sensitive to kanamycin. *E. coli* with both drug resistance and shiga toxin-encoding genes may make high virulence strains which represent a great health hazard for consumers. In agreement with the obtained results, multidrug resistance profiles for *E. coli* serovars were also reported in many recent studies conducted in Ghana (Eibach et al., 2018), Korea (Kim et al., 2018), Peru (Ruiz-Roldán et al., 2018), USA (Davis et al., 2018), and Vietnam (Yamaguchi et al., 2018).

Conclusion

This study indicated contamination of cattle meat (chuck, round, and masseter muscle) with coliforms and *E. coli*. The contamination might be started during slaughtering, evisceration, and preparation through cross-contamination with carcass-contact surfaces, including cutting-boards, walls, and floors at slaughterhouses or butcher shops. Strains of STEC were isolated and identified in this study. Such strains had a multidrug resistance profile. Therefore, strict hygienic procedures should be followed to avoid or reduce carcass cross-contamination. In addition, proper handling and efficient cooking of meat are highly recommended by consumers to reduce the risk of human exposure to STEC.

Author contributions

W.S.D. designed the study and wrote the manuscript. W.S.D. and A.S.A. conducted the experiments. A.S.A. and W.R.E-G. revised the manuscript. W.R.E-G. collected the samples. A.E.E. performed statistical analysis and drafted the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

The authors declared no conflict of interest.

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