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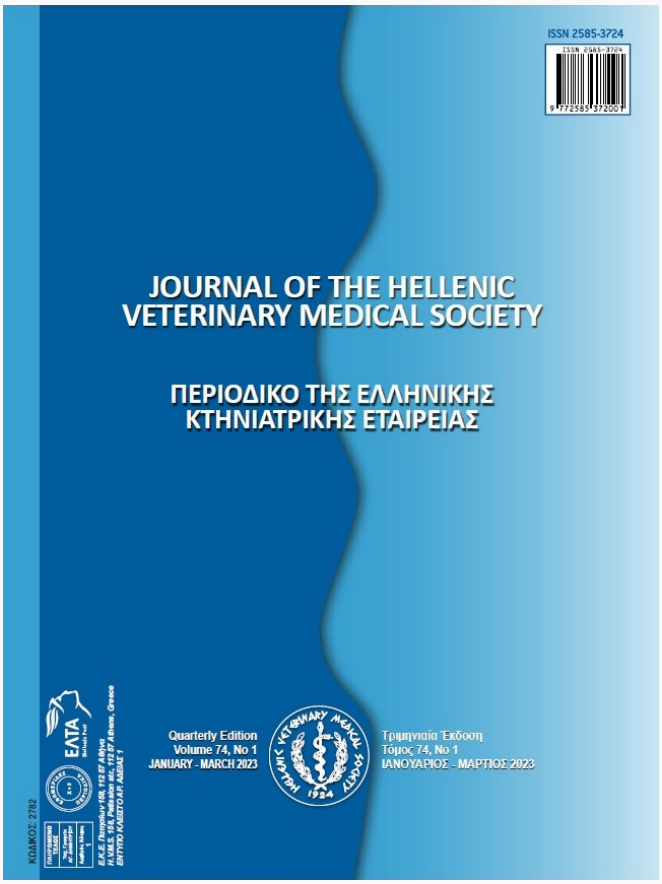

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Research article
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Phenotypic and genotypic aspects of sorbitol-negative *Escherichia coli* isolated from cattle

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ABSTRACT: *Escherichia coli* especially sorbitol-negative serogroups such as O157 that produce Shiga toxin (STEC) are involved in food poisoning with severity ranging from individual cases to outbreaks that threaten human and animal health. This study aimed to evaluate phenotypic and genotypic aspects of sorbitol-negative*E. coli* isolated from different sources.A total of 420 samples were collected from fecal swabs and raw milkof cattle from various locations in Kafr Elsheikh governorate, Egypt. The prevalence of *E. coli* isolates was 9.3% (39/420) as was determined by bacterial culturing.The phenotype of these isolates was serologically and molecularly determined, and the results showed an overall prevalence of *E. coli O157* of 23% (9/39) with a high incidence in fecal swabs samples. The multidrug resistance (MDR) phenotype as detected by antibiotic sensitivity test and confirmed by PCR revealed resistance to amoxicillin, clavulanic acid, vancomycin, ampicillin, and sulbactam with the detection of *blaTEM* and *blaSHV* MDR genes in 9 and 2 isolates, respectively.Virulence genes (*stx1*, *stx2*, *eaeA*) were also detected in 1, 9, 6 isolates, respectively.With these results, we could conclude that *E. coli O157* was identified in not only cattle fecal swab samples but also in their milk and subsequently this could threaten animal and human health.

Keywords: *E coli O157*; virulence factors;antibiotic resistance; STEC; milk

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INTRODUCTION

Escherichia coli is a member of the family Enterobacteriaceae, facultatively anaerobic, Gram-negative short rods and counted a common occupant of the stomach of warm-blooded animals, together with man, but similarly be in the water, soil, or other environments due to fecal contamination (Bujňáková *et al.*, 2021). Based on virulence, the enteric *E. coli* is categorized into enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxigenic (VTEC), enterohemorrhagic [(EHEC, which is a subgroup of Shiga toxin-producing *E. coli* (STEC)],diffusely adherent (DAEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and necrotoxic (NTEC) (Hammerum and Heuer, 2009). Among the different serogroups, *E. coli O157* is a foodborne pathogen that can cause hemorrhagic colitis (HC), hemolytic-uremic syndrome (HUS), watery and/or bloody diarrhea, and thrombotic thrombocytopenic purpura (TTP) in infected human (Bandyopadhyay *et al.*, 2011, Shahreza *et al.*, 2017). Food animals such as cattle are the main animal reservoir of *E. coli O157* (Ferens and Hovde, 2011, Yan *et al.*, 2011). Humans can be infected by the consumption of either contaminated animal milk (Lika *et al.*, 2021) or water, and food contaminated with food animal and all companion animal fecal matter (Amézquita-López *et al.*, 2016, Puvača and de Llanos Frutos, 2021). Moreover, STEC was considered as one of the normal flora inhabitants of the animal intestine (Hammerum and Heuer, 2009, Ibekwe *et al.*, 2011). This increases the prevalence of human infection.

E. coli O157 pathogenicity depends mainly on virulence factors such as Shiga toxin and intimin (Ojo *et al.*, 2010, Shahreza *et al.*, 2017). Intimin that is encoded by the *eaeA* gene is essential for attaching, while Shiga toxins are responsible for binding to the glycolipid globotriaosylceramide (Gb3) on the target cell surface preventing protein synthesis and leading to the death of infected cells (Bandyopadhyay *et al.*, 2011, Amézquita-López *et al.*, 2016, Mir *et al.*, 2016, Shahreza *et al.*, 2017).

This study was conducted to investigate phenotypic and genotypic aspects of sorbitol negative *E. coli* isolates collected from cattle fecal swabs and milk samples.

MATERIALS AND METHODS

Isolation and identification of E. coli

A total of 420 samples were collected from diarrheic cattle's fecal swabs and raw milk in the Kafr-El-Sheikh governorate. Fecal swab (230) and milk (190) samples were collected from private veterinary clinics. Collected samples were first enriched in tryptic soy broth for 24 h, then 100 µl were cultured onto Eosin Methylene Blue agar (Difco) to presumably detect *E. coli* (green-metallic colonies), and finally, these colonies were plated onto Sorbitol MacConkey agar (Difco) to determine sorbitol non-fermenting bacteria (colorless colonies, n = 39). The latter were plated on Tryptic Soy agar and were identified morphologically by Gram staining and biochemically by sugar fermentation, indole production, methyl-red, and Voges Proskauer tests (Quinn *et al.*, 2002).

Serological identification of E. coli O157

E. coli isolates were serotyped in Animal Health Research Institute, Dokki, Giza using standard monovalent *E. coli O157* antisera as previously described (Edwards and Ewing, 1972).

Antimicrobial drug sensitivity test

The sensitivity test was performed using the disk diffusion method which was performed using Muller-Hinton agar and 16 antibiotic disks (listed in Table 3) as previously described (Jorgensen JH *et al.*, 1997).The antibiotic sensitivity of isolates was measured based on the diameters of growth inhibition zones. The results were interpreted based on the Clinical and Laboratory Standards Institute Guidelines (Clinical and Institute, 2011).

Polymerase chain reaction (PCR)

Genomic DNA was isolated from *E. coli* isolates using a commercially available kit (QIAamp, Qiagen, GmbH, Germany) following the manufacturer's instructions and as previously detailed (Allam *et al.*, 2019). Polymerase chain reaction (PCR) was used to detect virulence (*stx1*, *stx2*, *eaeA*) and drug resistance (*blaTEM* and *blaSHV*) genes of *E. coli* using specific primers (Table 1). A PCR reaction mixture (30 µl) was set and included 5 µl genomic DNA, 15µl 2x Master mix (Emerald Amp GT, Takara, Japan), 1 µl from each primer (20 pmol), and 8 µl nuclease-free water. The PCR thermal cycler (Applied biosystem 2720) was set with the following thermal conditions: 94°C/5 min (initial denaturation), followed by 35 cycles of 94°C/30 sec (denaturation), 58°C for *stx1*, *stx2*, 51°C for *eaeA*, 54°C for *blaTEM* and *blaSHV* (annealing temperature for 40 sec), 72°C/45 sec (extension), followed by 72°C/10 min (final extension). Agarose gel

Table 1. Primers sequences and amplicon sizes.

Gene	Forward	Reverse	Size (bp)	Reference
Stx1	ACACTGGATGATC TCAGTGG	CTGAATCCCCCTC CATTATG	614	(Dipineto *et al.*, 2014)
Stx2	CCATGACAACGGA CAGCAGTT	CCTGTCAACTGAGC AGCACTTTG	779	
eaeA	ATGCTTAGTGCTGG TTTAGG	GCCTTCATCATTTCG CTTTC	248	(Bisi-Johnson *et al.*, 2011)
blaTEM	ATCAGCAATAAAC CAGC	CCCCGAAGAACGTT TTC	516	(Colom *et al.*, 2003)
blaSHV	AGGATTGACTGCC TTTTTG	ATTTGCTGATTTCGC TCG	392	

(1.5%) electrophoresis (Applichem, GmbH, Germany) with a DNA 100 bp marker (Qiagen, GmbH, Germany) was applied to determine PCR product size. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

RESULTS AND DISCUSSION

Prevalence of E. coli O157

Morphological and biochemical identification revealed the presence of a total of 39 positive *E. coli* isolates recovered from 420 samples with an overall prevalence of 9.3% (Table 2). The prevalence of the positive isolates distributed based on their source was 12.2% (28/230) from diarrheic cattle's fecal swabs and 5.8% (11/190) from raw milk. Based on serological identification, a total of 9 positive *E. coli* O157 isolates recovered from the positive 39 *E. coli* isolates with a prevalence of 23.1% (Table 2). The distribution of *E. coli* O157 based on the source of the samples was 28.6% (8/28) from diarrheic cattle's fecal swabs and 9.1% (1/111) from raw milk.This infers that the recovery rate of *E. coli* O157 isolates varies according to the sample source. We found a higher incidence in diarrheic cattle's fecal swabs than raw milk. *E. coli O157* recovery rate was close to that obtained by Hussein and Bollinger (2005) but higher than that reported by Cernicchiaro *et al.* (2012) who reported a recovery rate of 27.8% and 19%, respectively in cattle fecal matter. In contrast, Selim *et al.* (2013) did not find *E. coli* O157 in cattle fecal samples but rather recovered *E. coli* O26. Regarding the raw milk samples, our results agree with Mohamed *et al.* (2003) who found an *E. coli* O157 recovery rate of 7.1%. However, Abdul-Raouf *et al.* (1996) reported a lower prevalence rate of 6% in raw milk.

Antimicrobial resistance profile and antibiotic resistance genes of E. coli O157

The massive use of antimicrobial drugs in animal farms is one of the most causative factors responsible for the development of microorganisms that resist antibiotics (Darwish *et al.*, 2013). The antibiotic resistance profile of the isolated *E. coli* O157 serogroups was presented in Table 3. *E. coli O157* isolates were 100% sensitive toTetracycline, Ciprofloxacin, Ofloxacin, Norfloxacin, and Azithromycin. *E. coli O157* isolates showed high to moderate sensitivity to Cloxacillin, Ampicillin, Cefoxitin, Sulfamethoxazole, Doxycycline, Cefoperazone, Erythromycin, and Amikacin. However, *E. coli O157* isolates showed 100% resistance against Amoxicillin + Clavulanic acid, Ampicillin + Sulbactam, and Vancomycin. These results are consistent with those obtained bySelim *et al.* (2013) who found high resistance of *E. coli* O157 isolated from stool to Amoxicillin + Clavulanic acid, Ampicillin + Sulbactam, and Vancomycin. The presence of antibiotic-resistant strains of *E. coli* signifies the main health threat and indicates that Amoxicillin

Table 2. Prevalence of E. coli in the examined samples

Sample	No of samples	No. of *E. coli* positive samples	Prevalence (%*)	No. of *E. coli* O157 positive samples	Prevalence (%**)
Fecal swab	230	28	12.2	8	28.6
Milk	190	11	5.8	1	9.1
Total	420	39	9.3	9	23.1

%*: No of *E. coli* positive samples / No of examined samples.
%**: No of *E. coli* O157 positive samples / No of *E. coli* positive samples.

+ Clavulanic acid, Ampicillin + Sulbactam, and Vancomycin could have limited use. Having in mind high antimicrobial resistance towards mentioned antibiotics, usage of natural alternatives or essential oils qualitative and quantitative mixtures could be promising solution (Mileva *et al.*, 2020, Khan *et al.*, 2022).

Table 3. Results of antimicrobial sensitivity test for E. coli O157 .

Antimicrobial agent	Resistant		Sensitive	
	No.	%	No.	%
Cloxacillin	2	22.2	7	77.8
Ampicillin	3	33.3	6	66.7
Cefoxitin	1	11.1	8	88.9
Cefotaxime	0	0	9	100
Sulfamethoxazole	2	22.2	7	77.8
Amoxicillin + Clavulanic acid	9	100	0	0
Ampicillin + Sulbactam	9	100	0	0
Tetracycline	0	0	9	100
Doxycycline	2	22.2	7	77.8
Cefoperazone	1	11.1	8	88.9
Ciprofloxacin	0	0	9	100
Ofloxacin	0	0	9	100
Norfloxacin	0	0	9	100
Erythromycin	1	11.1	8	88.9
Azithromycin	0	0	9	100
Amikacin	2	22.2	7	77.8
Vancomycin	9	100	0	0

Beta-lactams are extensively utilized in the treatment of diseases caused by *E. coli* in animals. However, their massive use could result in the development of Beta-lactams resistant strains of *E. coli*. Transmission of antibiotic resistance genes, such as TEM and SHV β-lactamase-encoding genes (*blaTEM* and *blaSHV*) to humans should be considered when farm animals are infected with MDR bacterial strains (Manges *et al.*, 2007). In the present study, we detected *blaTEM* in all *E. coli* O157 isolates (n = 9) with a prevalence rate of 100% (Figs. 1, 2). However, *BlaSHV* was detected only in 2 isolates with a prevalence rate of 22.2%. Based on the source of the sample, the fecal samples showed a higher incidence of 100% (8/8) for *blaTEM*and 100% (1/1) for *BlaSHV* than milk samples which showed 25% (2/8) for *blaTEM* while no *BlaSHV* was detected. This implies that the results of the sensitivity test agree with that of PCR and confirm the presence of antibiotic resistance genes. In support, other studies also detected *blaTEM* and *BlaSHV* in animal fecal samples but with different prevalence of 89% and 42.9%, respectively (Rocha-Gracia *et al.*, 2014). *E. coli O157* expressing *blaTEM* and *BlaSHV* constitute multidrug resistance (Hosu *et al.*, 2021). With these findings, we could conclude that many diarrheic animals harbor β-lactam-resistant *E. coli* which threatens the health of humans and animals.

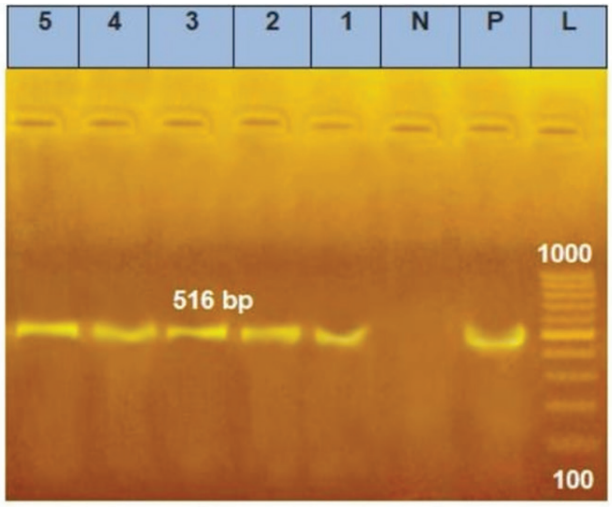

Fig. 1. Agarose gel electrophoresis of PCR amplified products of the antibiotic resistance *blaTEM* gene.Lane L, DNA marker (100 bp); P, positive control; N, negative control; *E. coli* O157 isolates from cattle fecal samples (lanes 1- 4) and raw milk (lane 5). The size in base pairs (bp) of each PCR product is indicated above the bands.

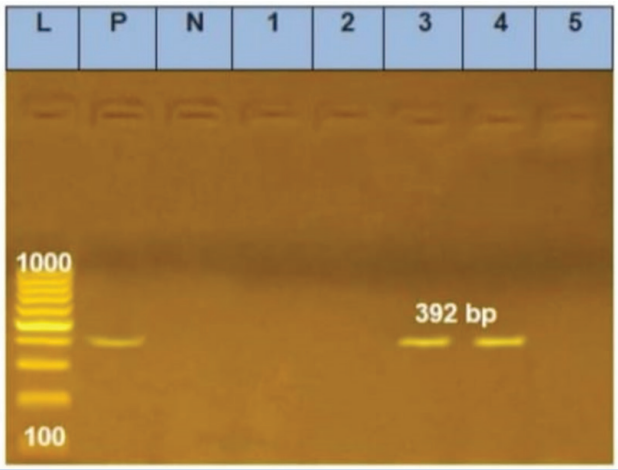

Fig. 2. Agarose gel electrophoresis of PCR amplified products of antibiotic resistance blaSHV gene. Lane L, DNA marker (100 bp); P, positive control; N, negative control; E. coli O157 isolates from cattle fecal samples (lanes 1- 4) and raw milk (lane 5). The size in base pairs (bp) of each PCR product is indicated above the bands.

Virulence genes

Shiga toxins and Intimin play a crucial role in the pathogenicity of STEC (Ojo *et al.*, 2010). Shiga toxins participate in the attachment of bacterial holotoxin to the cell membrane of the host cells and inhibit protein synthesis leading to the destruction of affected cells (Newton *et al.*, 2009). The Intimin, which is encoded by the *eaeA* gene, participates in the development of

Attaching/Effacing (A/E) lesion which destroys the microvilli and helps bacterial colonization into the lining epithelia of the intestine leading to the hemolytic uremic syndrome in humans (Woodward *et al.*, 2003).

In the present study, PCR was used to detect *stx1, stx2,* and *eaeA* virulence genes in the 9 pathogenic *E. coli* O157 isolates (Figs. 3-5).The incidence rates were 11.1% (1/9) for the *stx1*gene, 100% (9/9) for the *stx2* gene, and 66.6% (6/9) for the *eaeA* gene. Both *stx2* and *eaeA* virulent genes were detected in fecal samples (8/8, 5/8) and raw milk (1/1), respectively. However, *Stx1* was only detected in fecal samples.

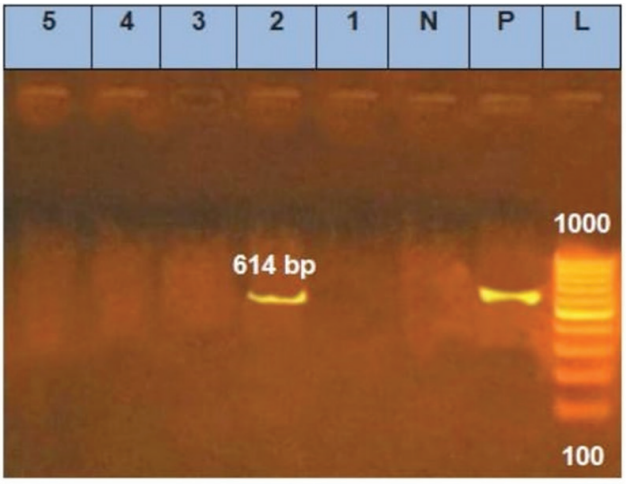

Fig. 3. Agarose gel electrophoresis of PCR amplified products of virulence *sxt1* gene. *E. coli* O157 isolates from cattle fecal samples. Lane L, DNA marker (100 bp); P, positive control; N, negative control; *E. coli* O157 isolates from cattle fecal samples (lanes 1- 4) and raw milk (lane 5). The size in base pairs (bp) of each PCR product is indicated above the bands.

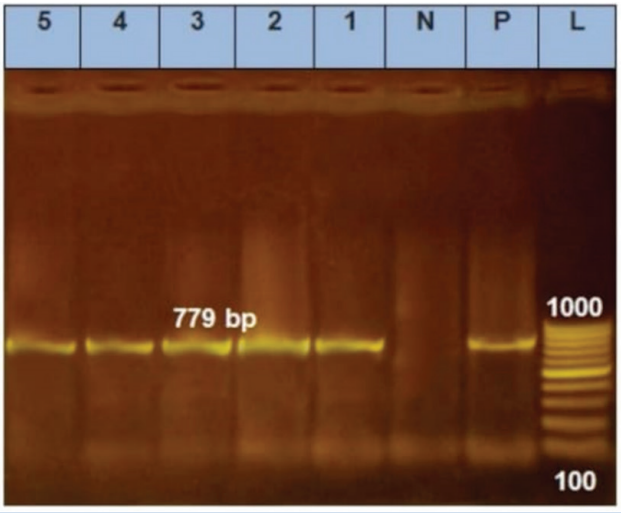

Fig. 4. Agarose gel electrophoresis of PCR amplified products of virulence sxt2 gene. lane L, DNA marker (100 bp); P, positive control; N, negative control; E. coli O157 isolates from cattle fecal samples (lanes 1- 4) and raw milk (lane 5). The size in base pairs (bp) of each PCR product is indicated above the bands.

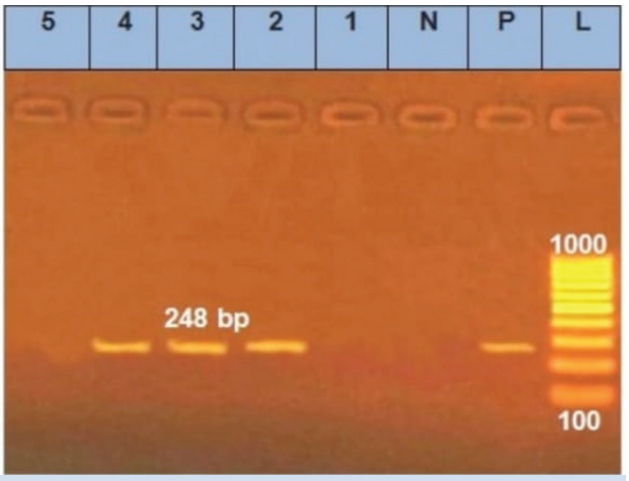

Fig. 5. Agarose gel electrophoresis of PCR amplified products of virulence eaeA gene. Lane L, DNA marker (100 bp); P, positive control; N, negative control; E. coli O157 isolates from cattle fecal samples (lanes 1- 4) and raw milk (lane 5). The size in base pairs (bp) of each PCR product is indicated above the bands.

Our findings regarding the prevalence of *stx1* and *stx2* genes in fecal samples of cattle are compatible with those obtained by Barkocy-Gallagher *et al.* (2001) and Khanjar and Alwan (2014). Similarly,stx1 and stx2 genes were detected in 38% of *E. coli* isolates of raw milk and beef meat contaminated with feces of animals (Shahzad *et al.*, 2013). Moreover, Elder *et al.* (2000) detected *sxt1* and *sxt2* in beef and milk samples with prevalence rates of 1.4 and 41.2%, respectively. Similar to our results, another study also found a higher prevalence rate of *sxt2* (20%) than *stx1* (3%) in human clinical cases (Ostroff *et al.*, 1989).This is also supported by the findings of Oporto *et al.* (2008) who found that *E. coli* O157 isolates carried *stx2* and *eaeA* but only a few isolates had *stx1*. However, Himi *et al.* (2015) did not find *stx1* in all examined *E. coli* serogroups. It is well known that *stx2* is 1000 times more cytotoxic than *stx1* and therefore it is associated with many diseases in humans (Mir *et al.*, 2016).

Some animals harbor the Shiga toxin-producing *E. coli* O157 serotype in their intestine as a part of normal microflora (Fairbrother and Nadeau, 2006, Hammerum and Heuer, 2009, Ibekwe *et al.*, 2011) and therefore these animals could act as a reservoir for *E. coli* O157 with a high possibility for human infection (Ferens and Hovde, 2011, Yan *et al.*, 2011). This serotype can transmit from an animal into humans through consumption of contaminated and raw food and milk (Karch *et al.*, 2005, Doma *et al.*, 2020) causing severe diseases including hemorrhagic diarrhea and renal failure which can lead to deaths especially in young children, old and immunocompromised

patients (Bandyopadhyay *et al.*, 2011, Shahreza *et al.*, 2017). Although *E. coli* O157 is not pathogenic to animals, an outbreak of fatal meningoencephalitis and septicemia in one-month-old goats caused by this serotype has been recorded (Filioussis *et al.*, 2013)

CONCLUSIONS

The presence of Shiga toxin-producing *E. coli* O157 in fecal and raw milk samples of cattle threatens public health as cattle could serve as themain reservoir for transmission of this pathogenic serotype to humans. This is of particular importance because *E. coli* O157 is a member of cattle microflora that rarely cause diseases to these animals and so the possibility of transmission to humans (via fecal-oral route) is higher. Eating raw food or milk contaminated with cattle feces containing *E. coli* O157 could lead to STEC foodborne diseases in humans. Thus, attention should be given to biosecurity control measures in cattle farms to prevent the transmission of *E. coli* O157 from animals to humans. Additionally, *E. coli* O157 had multiple extended-spectrum β-lactamase genes that showed a remarkable resistance to some antibiotics commonly utilized in animal farms. Hence, many investigations are required to choose appropriate antibiotics and avoid misuse of antibiotics and subsequently reduce the spread of antibiotic resistance genes between different bacterial populations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

ABDUL-RAOUF UM, AMMAR MS and BEUCHAT LR.Isolation of Escherichia coli O157 :H7 from some Egyptian foods. Int J Food Microbiol 29, 423-426, 1996.

ALLAM SA, MOSTAFA NY, KIRRELLA GAK, ELEIWA NZ and EL-MAGD MA.Molecular detection of Inva and Hila virulent genes in salmonella serovars isolated from fresh water fish. Slov Vet Res 56, 693–698, 2019.

AMÉZQUITA-LÓPEZ BA, QUIÑONES B, SOTO-BELTRÁN M, LEE BG, YAMBAO JC, LUGO-MELCHOR OY and CHAIDEZ C.Antimicrobial resistance profiles of Shiga toxin-producing Escherichia coli O157 and Non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. Antimicrobial Resistance and Infection Control 5, 2016.

BANDYOPADHYAY S, MAHANTI A, SAMANTA I, DUTTA T, GHOSH MK, BERA A, BANDYOPADHYAY S and BHATTACHARYA D.Virulence repertoire of Shiga toxin-producing Escherichia coli (STEC) and enterotoxigenic Escherichia coli (ETEC) from diarrhoeic lambs of Arunachal Pradesh, India. Tropical animal health and production 43, 705-710, 2011.

BARKOCY-GALLAGHER GA, ARTHUR TM, SIRAGUSA GR, KEEN JE, ELDER RO, LAEGREID WW and KOOHMARAIE M.Genotypic analyses of Escherichia coli O157 :H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States. Appl Environ Microbiol 67, 3810-3818, 2001.

BISI-JOHNSON MA, OBI CL, VASAIKAR SD, BABA KA and HATTORI T.Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens 3, 9, 2011.

BUJŇÁKOVÁ D, KARAHUTOVÁ L and KMEŤ V.Escherichia coli Specific Virulence-Gene Markers Analysis for Quality Control of Ovine Cheese in Slovakia. Microorganisms 9, 1808, 2021.

CERNICCHIARO N, PEARL DL, MCEWEN SA, HARPSTER L, HOMAN HJ, LINZ GM and LEJEUNE JT.Association of wild bird density and farm management factors with the prevalence of E. coli O157 in dairy herds in Ohio (2007-2009). Zoonoses Public Health 59, 320-329, 2012.

CLINICAL and INSTITUTE LS.Performance standards for antimicrobial susceptibility testing: Twenty-First Informational Supplement. CLSI Document M100-S21, 2011.

COLOM K, PÉREZ J, ALONSO R, FERNÁNDEZ-ARANGUIZ A, LARIÑO E and CISTERNA R.Simple and reliable multiplex PCR assay for detection of blaTEM, bla (SHV) and blaOXA-1 genes in Enterobacteriaceae. FEMS Microbiol Lett 223, 147-151, 2003.

DARWISH WS, ELDALY EA, EL-ABBASY MT, IKENAKA Y, NAKAYAMA S and ISHIZUKA M.Antibiotic residues in food: the African scenario. Japanese Journal of Veterinary Research 61, S13-S22, 2013.

DIPINETO L, RUSSO TP, GARGIULO A, BORRELLI L, DE LUCA BOSSA LM, SANTANIELLO A, BUONOCORE P, MENNA LF and FIORETTI A.Prevalence of enteropathogenic bacteria in common quail (Coturnix coturnix). Avian Pathology 43, 498-500, 2014.

DOMA AO, POPESCU R, MITULEȚU M, MUNTEAN D, DÉGI J, BOLDEA MV, RADULOV I, DUMITRESCU E, MUSELIN F, PUVAČA N and CRISTINA RT.Comparative Evaluation of qnrA, qnrB, and qnrS Genes in Enterobacteriaceae Ciprofloxacin-Resistant Cases, in Swine Units and a Hospital from Western Romania. Antibiotics 9, 698, 2020.

EDWARDS PR and EWING WH.Identification of enterobacteriaceae. Identification of Enterobacteriaceae., 1972.

ELDER RO, KEEN JE, SIRAGUSA GR, BARKOCY-GALLAGHER GA, KOOHMARAIE M and LAEGREID WW.Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proceedings of the National Academy of Sciences of the United States of America 97, 2999-3003, 2000.

FAIRBROTHER JM and NADEAU E.Escherichia coli: on-farm contamination of animals. Rev Sci Tech 25, 555-569, 2006.

FERENS WA and HOVDE CJ.Escherichia coli O157 :H7: animal reservoir and sources of human infection. Foodborne Pathog Dis 8, 465-487, 2011.

FILIOUSSIS G, PETRIDOU E, KARAVANIS E, GIADINIS ND, XEXAKI A, GOVARIS A and KRITAS SK.An outbreak of caprine meningoencephalitis due to Escherichia coli O157 :H7. Journal of Veterinary Diagnostic Investigation 25, 816-818, 2013.

HAMMERUM AM and HEUER OE.Human health hazards from antimicrobial-resistant Escherichia coli of animal origin. Clin Infect Dis 48, 916-921, 2009.

HIMI HA, PARVEJ MS, RAHMAN MB, NASIRUDDIN KM, ANSARI WK and AHAMED MM.PCR Based Detection of Shiga Toxin Producing E. coli in Commercial Poultry and Related Environments. Turkish Journal of Agriculture - Food Science and Technology 3, 361, 2015.

HOSU MC, VASAIKAR SD, OKUTHE GE and APALATA T.Detection of extended spectrum beta-lactamase genes in Pseudomonas aeruginosa isolated from patients in rural Eastern Cape Province, South Africa. Scientific Reports 11, 7110, 2021.

HUSSEIN HS and BOLLINGER LM.Prevalence of Shiga toxin-producing Escherichia coli in beef cattle. J Food Prot 68, 2224-2241, 2005.

IBEKWE AM, MURINDA SE and GRAVES AK.Genetic Diversity and Antimicrobial Resistance of Escherichia coli from Human and Animal Sources Uncovers Multiple Resistances from Human Sources. PLoS ONE 6, e20819, 2011.

JORGENSEN JH, TURNIDE JD and JA W.Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods. In: Manual of Clinical Micro-biology, ASM Press, Washington, DC 4th ed., 1997.

KARCH H, TARR PI and BIELASZEWSKA M.Enterohaemorrhagic Escherichia coli in human medicine. Int J Med Microbiol 295, 405-418, 2005.

KHAN RU, FATIMA A, NAZ S, RAGNI M, TARRICONE S and TUFARELLI V.Perspective, Opportunities and Challenges in Using Fennel (Foeniculum vulgare) in Poultry Health and Production as an Eco-Friendly Alternative to Antibiotics: A Review. Antibiotics 11, 278, 2022.

KHANJAR AF and ALWAN MJ.Genotypic Study of Escherichia coli O157 :H7 Isolated from Stool Samples of Humans and Cattle. Int. J. of Adv. Res. 2, 12-18, 2014.

LIKA E, ROSIĆ M, COCOLI S, PUVAČA N, VUKOVIĆ G, KIKA TS and BURSIĆ V.Antimicrobial resistance of Staphylococcus aureus strains isolated from cow raw milk samples from Albania and Serbia. Mljekarstvo 71, 248-256., 2021.

MANGES AR, SMITH SP, LAU BJ, NUVAL CJ, EISENBERG JNS, DIETRICH PS and RILEY LW.Retail Meat Consumption and the Acquisition of Antimicrobial ResistantEscherichia coliCausing Urinary Tract Infections: A Case–Control Study. Foodborne Pathog Dis 4, 419-431, 2007.

MILEVA R, KARADAEV M, FASULKOV I, PETKOVA T, RUSENOVA N, VASILEV N and MILANOVA A.Oxytetracycline Pharmacokinetics After Intramuscular Administration in Cows with Clinical Metritis Associated with Trueperella Pyogenes Infection. Antibiotics 9, 392, 2020.

MIR RA, WEPPELMANN TA, ELZO M, AHN S, DRIVER JD and JEONG KC.Colonization of Beef Cattle by Shiga Toxin-Producing Escherichia coli during the First Year of Life: A Cohort Study. PLoS ONE 11, e0148518, 2016.

MOHAMED MEM, MOHAMED AA and GHARIB AA.Zoonotic and microbiological aspects of Escherichia coli O157 with special reference to its RAPD-PCR. . Vet. Med. J. Giza 51, 467-484, 2003.

NEWTON HJ, SLOAN J, BULACH DM, SEEMANN T, ALLISON CC, TAUSCHEK M, ROBINS-BROWNE RM, PATON JC, WHITTAM TS, PATON AW and HARTLAND EL.Shiga toxin-producing Escherichia coli strains negative for locus of enterocyte effacement. Emerg-

ing infectious diseases 15, 372-380, 2009.
OJO OE, AJUWAPE ATP, OTESILE EB, OWOADE AA, OYEKUNLE MA and ADETOSOYE AI.Potentially zoonotic shiga toxin-producing Escherichia coli serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. International Journal of Food Microbiology 142, 214-221, 2010.
OPORTO B, ESTEBAN JI, ADURIZ G, JUSTE RA and HURTADO A.Escherichia coli O157 :H7 and non-O157 Shiga toxin-producing E. coli in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses Public Health 55, 73-81, 2008.
OSTROFF SM, KOBAYASHI JM and LEWIS JH.Infections with Escherichia coli O157 :H7 in Washington State. The first year of statewide disease surveillance. Jama 262, 355-359, 1989.
PUVAČA N and DE LLANOS FRUTOS R.Antimicrobial Resistance in Escherichia coli Strains Isolated from Humans and Pet Animals. Antibiotics 10, 69, 2021.
QUINN P, MARKEY BK, CARTER M, DONNELLY W and LEONARD F 2002. Veterinary microbiology and microbial disease. Blackwell science.
ROCHA-GRACIA R, CORTÉS-CORTÉS G, ZARAIN P, BELLO F, MARTÍNEZ-LAGUNA Y and TORRES C.Faecal Escherichia coli isolates from healthy dogs harbour CTX-M-15 and CMY-2 β-lactamases. The Veterinary Journal 203, 12-18, 2014.
SELIM SA, AHMED SF, AZIZ MHA, ZAKARIA AM, KLENA JD and PANGALLO D.Prevalence and Characterization of Shiga-Toxin O157 :H7 and Non-O157 :H7 Enterohemorrhagic Escherichia Coli Isolated from Different Sources. Biotechnology & Biotechnological Equipment 27, 3834-3842, 2013.
SHAHREZA MHS, RAHIMI E and MOMTAZ H.Antibiotic resistance pattern of Shiga-toxigenic Escherichia coli isolated from ready-to-eat food stuffs. Bioscience Biotechnology Research Communications 10, 155-159, 2017.
SHAHZAD K, KHUSHI M, SHEIKH A, YAQUB T, RABBANI M, HUSSAIN T, ANJUM A and ANEES M.Isolation and molecular characterization of Shiga toxin producing E. coli O157 . Journal of Animal and Plant Sciences 23, 1618-1621, 2013.
WOODWARD MJ, BEST A, SPRIGINGS KA, PEARSON GR, SKUSE AM, WALES A, HAYES CM, ROE JM, LOW JC and LA RAGIONE RM.Non-toxigenic Escherichia coli O157 :H7 strain NCTC12900 causes attaching-effacing lesions and eae-dependent persistence in weaned sheep. Int J Med Microbiol 293, 299-308, 2003.
YAN Y, SHI Y, CAO D, MENG X, XIA L and SUN J.Prevalence of Stx phages in environments of a pig farm and lysogenic infection of the field E. coli O157 isolates with a recombinant converting Phage. Curr Microbiol 62, 458-464, 2011.