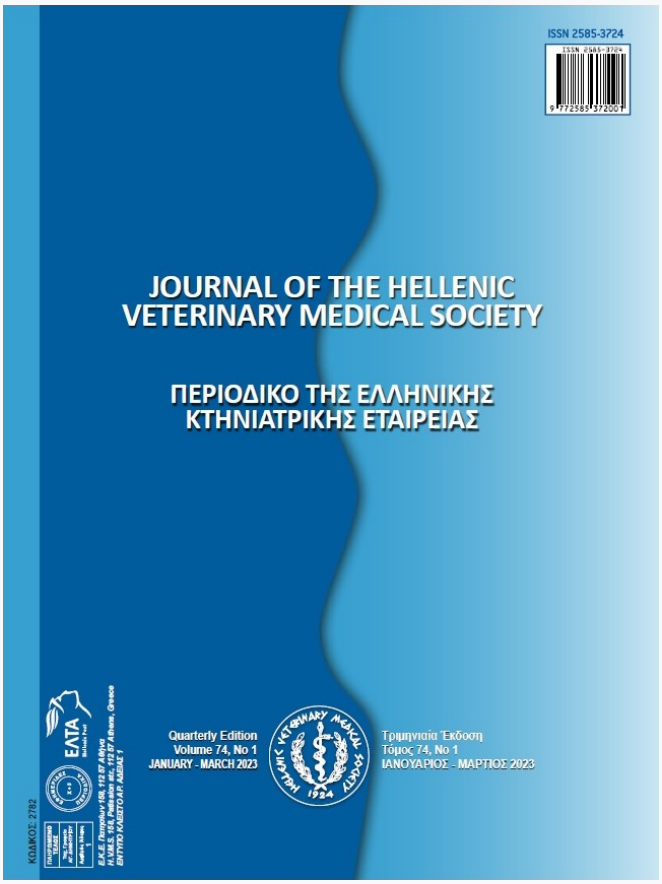
Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 74, Αρ. 1 (2023)



Antibiotic resistant shiga toxin producing Escherichia coli isolates from milk and milk products

AMK Sobeih, AA Moawad, T Sharshar

doi: 10.12681/jhvms.29111

Βιβλιογραφική αναφορά:

Sobeih, A., Moawad, A., & Sharshar, T. (2023). Antibiotic resistant shiga toxin producing Escherichia coli isolates from milk and milk products. *Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας*, *74*(1), 5283–5288. https://doi.org/10.12681/jhvms.29111 (Original work published 11 Απρίλιος 2023)

J HELLENIC VET MED SOC 2023, 74 (1): 5283-5288
ΠΕΚΕ 2023, 74 (1): 5283-5288

Research article
Ερευνητικό άρθρο

Antibiotic resistant shiga toxin producing *Escherichia coli* isolates from milk and milk products

A.M.K Sobeih[1], A.A. Moawad[2], T.A. Sharshar[2*]

[1]*Department of Food Control, Faculty of Veterinary Medicine, Kafrelsheikh university, Egypt*

[2]*Department of Bacteriology, mycology and immunology, Faculty of Veterinary Medicine, Kafrelsheikh university, Egypt*

ABSTRACT: A total of450 samples of milk and milk products (50 each of raw buffalo milk; raw cow milk; Karish, Talaga, and Roquefort cheeses; yogurt; Rayeb; condensed milk; and sour cream) were randomly purchased from various markets inKafr El-Sheikh for isolation and identification of Shiga toxin producing *Escherichia coli*(STECs). We found the pathogen in 60% of buffalo milk samples, 80% of cow milk, 30% of Karish, 10% of Roquefort, 12% of Talaga, 20% of yogurt, 6% of Rayeb and 22% of sour cream, but none in condensed milk. Fifty of suspected colonies were serologically identified, using *E.coli* O157:H7 kits; and confirmed in 69.2%, 53.8%, 60%, 16.7% and 20% isolates of buffalo milk, cow milk, Karish, Talaga, and yogurt, respectively, while Roqufort, Rayeb, and sour cream were negative. Using polymerase chain reaction assays, 21 confirmed isolates were examined, and 44.4, 71.4, 66.7, 100, and 100% of isolates of buffalo milk, cow milk, Karish, Talaga, and yogurt respectively, were positive for *rfbE* gene; while 55.6, 42.8, 66.7, 100, and 100 % isolates of buffalo milk, cow milk, Karish, Talaga, and yogurt respectively, were positive for bla_{TEM}, but all isolates were negative for bla_{CMY2}. Consequently, raw milk and most dairy products including fermented products, were possible sources of *E.coli* O157:H7food poisoning, so the implementation of strict hygienic measures throughout the manufacture and retail of milk products is essential.

Key words: Shiga toxin producing *E. coli*; antibiotic resistant genes, bla_{TEM} and bla_{CMY2}.

Corresponding Author:
T.A. Sharshar, Department of Bacteriology, mycology and immunology, Faculty of Veterinary Medicine, Kafrelsheikh university, Egypt.
E-mail address: tasneemsharshar9gmail.com

Date of initial submission: 01-01-2022
Date of acceptance: 09-05-2022

INTRODUCTION

Shiga toxin producing *Escherichia coli* (STECs) is a serious class of pathogenic microbes which can cause zoonosis.*E.coli*O157 is the most frequent serotype (Franz, 2007). STECs include multiple strains, producing Shiga toxin (stx), and have common mechanisms of pathogenesis, entry, and attachment to the intestinal epithelium. However, the formation of Stx alone, without adherence, doesn't cause severe infections (FAO/WHO, 2018). Some members of the STECs group have public health significance, due to their association with sporadic cases and outbreaks of food-borne diseases in humans,the symptoms of which may vary from diarrhea, that may lead to hemorrhagic colitis and hemolytic uremic syndrome (Pennington, 2010). There are four major factors responsible for virulence of STECs: two phage encoded cytotoxins, Shiga toxin1 (*stx1*) and Shiga toxin2 (*stx2*), the protein intimin (*eae*), and entero-hemorrhagic *E.coli* hemolysin (*ehly*) (Law, 2000).

The inappropriate use of antimicrobials in humans and in food producing animal farms is the major cause of the development of bacterial antimicrobial resistance(AR) (FDA, 2009). Antibiotic resistant bacteria may occur spontaneously, or develop under certain circumstances, in the clinical effluents, dung, and farms. Individuals can then be infected through the ingestion of contaminated meat, milk, or other products, contaminated water supplies, or directly by handling carrier cases (Forsberg et al., 2012; Losada et al., 2016). STECs that are able to resist antimicrobials such as B-lactams, aminoglycosides, carbapenems, cephalosporin, erythromycin, phenicols, streptomycin, sulpha drugs, and tetracycline have been detected in both livestock and humans in many countries,both well developed,such as US, Brazil, and Mexico, or less developed, such as Egypt, and India (Ahmed and Shimamoto, 2015; Amezquita Lopez et al., 2016, Garcia et al., 2011; Mahanti et al., 2013). Considerable research has reported increased antibiotic resistance in STECs, particularly to promising antibiotics such as azithromycin. The existences of genes responsible for antimicrobial resistance carried by mobile genetic elements enhances the probability of transmission antibiotic resistance in STECs and reduce their therapeutic value for the treatment of human infections**.** (Mir and Kudva 2019)reported that cattle are a reservoir for STECs and recorded antibiotic resistant STECs from feedlot cattle in Canada, abattoirs in Ireland, beef and milk products in Egypt, and pork and poultry meat in South Korea**.**Antibiotic resistant strains of microbes caused more symptoms for longer periods of time than their antibiotic sensitive equivalents. Many studies have identified antibiotic resistance as rapidly evolved over the last few decades, representing significant threat to public health in the current century (Munita and Arias, 2016).

The identification of resistance genes of bacteria is fundamental to lowering cost of treatment. In this study we aimed to identify STECs strains from raw buffalo and cow milk, and other milk products collected from several markets in Kafr El-Sheikh governorate. Isolates were identified serologically against O157 and H7antisera. The identification of the isolates was confirmed by recognizing the characteristic *rfbE* gene for*E.coli*O157, and then tested for presence of the antibiotic resistance genes bla_{TEM} *and* bla_{CMY2} using PCR.

MATERIALS AND METHODS

Sample collection:

total of 450 milk and dairy product samples were purchased from several markets in the Kafr El-Sheikh governorate, Egypt, between November 2018 and February 2021. Among them, 100 raw milk samples (50 each of cow and buffalo milk), 150 cheeses (50 each of Karish, Talaga and Roquefort), 100 fermented milks (50 each of yogurt and Rayeb), 50 condensed milks and 50 sour cream samples were randomly collected under aseptic condition in sterile containers, or in their packages, kept in an ice box at 4°C, and delivered to the laboratory with minimum delay, to be examined immediately.

Karish cheese is described as soft unripen, fresh, skim milk cheese (EOSQ 2005a)

Talaga cheese is described asoft cheese obtained from pasteurized milk, preserved in brine and kept in refrigerator, (EOSQ 2005b).

Examination of milk samples for detection of heat treatment:

All milk samples were tested using Storch's test (APHA, 2004) to exclude samples shown to be heated above 80°C.

Sample preparation:

Direct plating of samples cannot detect exhausted bacteria that are unable to remain alive in selective media (Chapman et al., 1994). To increase the possibility of detection, samples can be inoculated into

Table 1 Target genes primer sequences

Primer Sequence	Amplified product	Reference	
bla_{TEM}	ATCAGCAATAAACCAGC	516 bp	Colom *et al*., 2003
	CCCCGAAGAACGTTTTC		
CIT (*blaCMY2*)	TGG CCA GAA CTG ACA GGC AAA	462 bp	Pérez-Pérez and Hanson, 2002
	TTT CTC CTG AAC GTG GCT GGC		
E. coli O157 rfbE	GTAAATATGTGGGAACATTTGG	134 bp	Heijnen and Medema, 2006
	GGCCTTTAAAATGTAAACAACGG		

nonselective enrichment broth such as buffered peptone water (Foster et al., 2003), or universal pre-enrichment broth such as Trypticase soya broth, or nutrient broth (NB) (Jiang et al., 1998).

Ten milliliter of each raw milk sample was injected into 90 ml NB(Merck, Darnstadt, Germany) and kept at 32°C for 24 h. Twenty-five grams of each milk product sample was homogenized with 255 ml NB for 2 min using stomacher and then incubated, at 37°C for 24 h. One milliliter of incubated NB was blended with 9ml of MacConkey broth (Merck) and held at 37°C for 24 h.

Conventional culture and plating on specific selective media.

Since the number of bacteria required to elicit human infection is low, the use of enrichment media is required to screen food for the presence of these organisms (De Boer and Heuvelink, 2000). About one loop of each pre-enriched broth was spread on the surface of MacConkey's agar plates with Eosin Methylene Blue (EMB). After incubation at 37°C for 18-24 h, colonies showing lactose fermentation (pink to dark pink) on MacConkey agar (Trepeta and Edberg, 1984) or greenish metallic sheen on EMB (Leininger et al., 2001) were selected and re-streaked on Cefixime tellurite Sorbitol MacConkey agar (CT-SMAC) as a specific selective media, and held at 37°C for 24h. Colorless (sorbitol negative) growth was characteristic of colonies with O157 STECs(March and Ratnam, 1986; De Boer and Heuvelink, 2000). These presumptive *E.coli* O157 colonies were purified and were serologically identified.

Serological identification:

Fifty purified isolates were identified by serological tests using slide agglutination reactions against O157 and H7 antisera, with sets of rapid diagnostic *E.coli* antisera (Denka Seiken Co., Takasaki-city, Japan) to confirm *E.coli* **(**according to Kok et al., 1996). Only strong agglutination observed within 1 min in the reaction was regarded as positive. While delayed or weak agglutination was regarded as negative.

Polymerase chain reaction

Twenty one identified *E.coli* isolates were subjected tPolymerase chain reaction(PCR) assays to identify the characteristic *rfbE* gene of *E.coli* O157 (Franz et al., 2007).This gene codes for the lipopolysaccharide O side chain of *E.coli* O157. PCR assays were used on the same 21 isolates to detect Beta-lactamase resistance, $bla_{TE}$and bla_{CMY2} genes.

The target genes primer sequences are shown in Table 1.

DNA was obtained using the **QIAamp DNA mini kit catalogue no 51304** prescript. Target genes were amplified in a thermocycler, the PCR products were separated using gel electrophoresis,and then visualized under U.V. The analysis of electrophoretic patterns of the product was performed as described by Sambrook et al., 1989 with modifications.

RESULTS

We examined a total of 450 raw milk and milk product samples, as shown in Table 2, on Sorbitol MacConkey agar plates.

Raw cow milk showed the highest percentage (60%) of STECs, and condensed milk samples had the lowest, as zero isolates were obtained from them.

The percentage of samples with STECs, from highest to lowest, were cow's milk, buffalo milk, Karish cheese, sour cream, yogurt, Talaga cheese, Roquefort cheese, Rayeb and condensed milk. A total of 26.7% is a high percentage of STECs in dairy products, as it is a common source of food especially for children.

Serological examination using slide agglutination tests of 50 isolates against O157 and H7 antisera was carried out.

Buffalo milk showed the highest percentage, then Karish cheese, then cow's milk, then yogurt and Talaga cheese.

Roquefort cheese and Rayeb samples were negative for this strain.

PCR is a confirmatory test, as it detects the presence of specific genes in the sample. PCR was done for the 21 positive isolates identified by serological examination.

Table 4 shows the results of the detection of the *rfbE* gene, which is specific for STECs, in the isolates. All 21 isolates contained this gene, at different percentages.

Identification of the bla_{TEM} gene in the 21 isolates. This gene produces antibiotic resistance to B-lactamase group in the isolates.

All samples contained this gene, at different percentages.

Results of PCR assay for identification of Bla_{CMY2}

Table 2 Incidence of *E. coli* isolated on Sorbitol MacConkey agar

Sample type		Number of examined samples	Positive samples	
			No.	% *
a-Raw milk	1-Buffalo milk	50	30	60%
	2-Cow milk	50	40	80%
b-Cheese	1-Karish	50	15	30%
	2-Roquefort	50	5	10%
	3-Talaga	50	6	12%
c-Fermented milk	1-Yogurt	50	10	20%
	2-Rayeb	50	3	6%
d-Condensed milk		50	0	0%
e-Sour cream		50	11	22%
Total		450	120	26,7%

* Percentage of the number of samples examined.

Table 3 Results of serological identification of 50 *E.coli* isolates against O157:H7 antisera

Sample type		No. of isolates examined	Positive isolates	
			No.	% *
a-Raw milk	1-Buffalo milk	13	9	69.2%
	2-Cow milk	13	7	53.8%
b-Cheese	1-Karish	5	3	60%
	2-Roquefort	5	0	0%
	3-Talaga	6	1	16.7%
c-Fermented milk	1-Yogurt	5	1	20%
	2-Rayeb	3	0	0%
Total		50	21	42%

* Percentage calculated according to the number. of isolates examined.

Table 4 Results of PCR assay for detection of the O157 *rfbE* gene.

Sample type		No. of isolates examined	Positive isolates	
			No.	% *
a-Raw milk	1-Buffalo milk	9	4	44.4%
	2-Cow milk	7	5	71.4%
b-Cheese	1-Karish	3	2	66.7%
	2-Talaga	1	1	100%
c-Fermented milk	1-Yogurt	1	1	100%
Total		21	13	61.9%

* Percentage calculated according to the number of isolates examined.

Table 5 Results of PCR assay for detection of the *bla*$_{TEM}$ gene

Sample type		No. of isolates examined	Positive isolates	
			No.	% *
a-Raw milk	1-Buffalo milk	9	5	55.6%
	2-Cow milk	7	3	42.8%
b-Cheese	1-Karish	3	2	66.7%
	2-Talaga	1	1	100%
c-Fermented milk	1-Yogurt	1	1	100%
Total		21	12	57.1%

* Percentage calculated according to the numberof isolates examined.

gene showed that all of the examined isolates proved negative.

DISCUSSION

STECs are significant cause of food borne disease and infections, which are associated with symptoms varying from mild to bloody diarrhea and hemolytic uremic syndrome associated with kidney failure (FAO/WHO, 2018).

In our study, 120 out of 450 (26.7%) of samples examined were positive for *E.coli* on CT-SMAC plates: 60% of raw buffalo milk, 80% of raw cow milk, 30% of Karish cheese 10% of Roquefort cheese, 12% of Talaga cheese 20% of yogurt,6% of Rayeb, and 22% of sour cream samples. STECs were not detected in condensed milk (Table 2). Elbastawesy et al. (2016) recorded the same percentage of *E.coli* O157:H7 in Karish cheese, and a higher rate in yogurt (31.5%) and sour cream (45.4%) samples on CT-SMAC plates. Garbi et al. (2016) recorded nearly similar percentages (25%) for all milk and dairy product samples with variable percentage for different samples. Their reported recovery rate of colonies suspected to be EHEC O157 in raw cow milk was 3 out of 28 (10.7%) samples, 7/28 (25%) of fermented raw milk, 9/21 (42.9%) of Massora and 3/10 (30%)of Ricotta samples (the latter two are fresh soft cheese). Using Trypton bile X glucouronide agar medium (TBX). Hassan et al. (2021) detected*E.coli* at a nearly similar percentage (75%) of raw milk and small scale yogurt (25%) samples but a higher percentage (62.5%) of the cream and (80%) of Karish cheese. However, none of the isolates could be identified serologically as O157.

Twenty one *E.coli* isolated from milk and its products were serologically identified and 9 out of 13 (69.2%), obtained from raw buffalo milk, 7/13 (53.8%), from raw cow milk, 3/5 (60%), from Karish cheese, one/6 (16.7%) fromTalaga cheese and one/5 (20%) from yogurt were confirmed using slide agglutination tests to be *E.coli* O157:H7 (table 3). EHEC O157:H7 was found in many of the raw milk and milk products examined, even fermented products,because of the ability of STECs to grow at wide range of temperatures from 7°C to 50°C and the ability of some strains to grow in acidic food down to pH of 4.4, and in foods with water activity (a_w) as low as 0.95 (FAO/WHO, 2018).

E.coli O157:H7 was not identified in any of the isolates obtained from Roquefort cheese, Rayeb, or sour cream samples. The mean value of a_w of Roquefort cheese is<0.95 (Satguer et al. 1986) this may be the cause that *E.coli* O157 failed to be revealed in isolates obtained from Roquefort cheese.

Hassan et al.; (2017) considered sour cream to be ripened or acidified cream, without any further specifications, so the pH of the samples they examined may be < 4.4, which prevents the growth of *E.coli* O157.

Our results differ from those of some other researchers, including Nahas et al. (2015) who examined 120 raw milk, Karish cheese, and cream samples gathered from the EL-Menofiya governorates, by serological. Ibrahim and Sobeih (2006) and Prencipe et al. (2010) could not isolate the organism from any of their samples of Karish and six Italian type) cheeses.

The results shown in table 4, of the PCR assays on 21 isolates, for the detection of the O157 *rfbE* gene that is specific to O157, showed positively rates of 4/9 (44.4%), for raw buffalo milk, 5/7 (71.4%), for raw cow milk, 2/3 (66.7%) forKarish cheese, 1/1 (100%) for Talaga cheese and 1/1 (100%) for yogurt. These isolates therefore contained food borne pathogens of significant public health significance especially the dairy products Karish, Talaga cheeses, and yogurt

ready for consumption.

PCR assays of the same 21 isolates were carried out to detect the presence of antibiotic resistance genes *bla*$_{TEM}$ and bla$_{CMY2}$. Out of 9, isolates obtained from raw buffalo milk, 7 from raw cow milk, 3 from Karish cheese, 1 from Talaga cheese and 1 from yogurt samples only 5 (55.6%), 3 (42.8%), 2 (66.7%), 1(100%) and 1(100%) samples respectively contained the *bla*$_{TEM}$ gene, and no isolates contained the bla$_{CMY2}$ gene (table 5).

Ali et al. (2016) identified the *bla*$_{TEM}$ gene using PCR analysis in 20 (55.56%) of *E.coli* strains obtained from milk sampled from animals with mastitis.

CONCLUSIONS

The predominance of the O157 serogroup was confirmed by the presence of the *rfbE* gene, a gene specific for O157, and was accompanied by the presence of an antibiotic resistance gene (*bla*$_{TEM}$) in samples of raw buffalo and cow milk, Karish and Talaga cheeses and yogurt. The presence of this pathogen presents a health risk to consumers of raw milk and raw milk products. Efficient heat treatment of raw milk before consumption or processing is therefore essential to control STECsinfections in humans. Attention should also be paid to the principles of hazard analysis and critical control point, to eliminate the public health hazard of STECs. It is also important to use disc diffusion tests and follow antibiotic prescription guidance, to reduce the threat of antimicrobial resistant STECs in raw milk and its products.

CONFLICT OF INTEREST

None declared by the authors.

REFERENCES

Ahmed AM, Shimamoto T (2015) Molecular analysis of multidrug resistance in shiga toxin producing *Escherichia coli* O157:H7 isolated from meat and dairy products. Int J Food Microbiol 193:68-73.

Ali T, Rahman S, Zhang L, Shahid M, Zhang S, Liu G, Gao J, Han B (2016) ESBL Producing *Escherichia coli* form cows suffering mastitis in China contain clinical class 1 Intergons with CTX_M Linked to ISCR1. Front Microbiol 7:1931.

Amézquita-López BA, Quiñones B, Soto-Beltrán M, Lee BG, Yambao JC, Lugo-Melchor OY, Chaidez C (2016) Antimicrobial resistance profiles of shiga toxin producing *Escherichia coli* O157 and non O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. Antimicrob Resist Infect Control 5:1.

APHA (American Public Health Association) (2004) Standard methods for the examination of dairy products, 17th ed. American public health Association. Washington DC, USA.

Cardozo MV, Nespolo N, Delfino TC, de Almeida CC, pizauro LJL, Valmorbida MK, Pereira N, de Avila FA (2021) Raw milk cheese as a potential infection source of pathogenic and toxogenic food borne pathogens. Food Sci Technol Campinas 41:355-358.

Chapman PA, Wright DJ, Siddons CA (1994) A comparison of immune magnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. J Med Microbiol 40:424-427.

Clinical Laboratory Standards Institute (CLSI) (2006) Performance standards for antimicrobial Disk susceptibility tests, Approved Standard-9th Ed. (M2-Ag), Clinical and laboratory standards Institute, Wayne, Pa, USA.

Colom K, Pèrez J, Alonso R, Fernández-Aranguiz A, Lariño E, Cisterna R (2003) Simple and reliable multiplex PCR assay for detection of bla_{TEM},bla_{SHV} and bla_{OXA-1} genes in Enterobacteriaceae. FEMS Microbiol Letters J 223:147-151.

Cortés P, Blanc V, Mora A, Dahbi G, Blanco JE, Blanco M, López C, Andreu A, Navarro F, Alonso MP, Bou G, Blanco J, Llagostera M (2010) Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl. Environ Microbiol 76:2799-2805.

De Boer E, Heuvelink AE (2000) Methods for the detection and isolation of shiga toxin-producing *Escherichia coli*. Symp Ser Soc Appl Microbiol. 88:133-143.

El Gamal AM, Abdou MS, E.bied NA, Salem NI (2019) Isolation and molecular identification of E.coli O157 from raw milk and some milk products in KafrElsheikh governorate. Anim Health Res J 3:283-289.

Elbastawesy AM, Deeb AMM, El-desoky NI (2016) Incidence of E.coli O157:H7 in some dairy products. Kafrelsheikh Vet Med 19:315-328.

Egyptian Organization for Standardization and Quality (EOSQ) (2005a) Soft cheese, Karish cheese, ES: 1008-4/2005.

Egyptian Organization for Standardization and Quality (EOSQ) (2005b) Soft cheese, Cold stored cheese, ES: 1008-5/2005.

El nahas AW, Mohamed HA, Elbarbary HA, Mohamed HS (2015). Incidence of E. coli in raw milk and its products. Benha Vet Med J 29:112-117.

FDA (2009) Summary report on antimicrobials sold or distributed for use in food producing animals. http://www.fda.gov/downloads/farindustry/userfees/animaldruguserfeeactadufa/UCm231851.pdf[accessed 25 February 2019]

Food and Agriculture organization of the United Nations World Health Organization (FAO/WHO) (2018). Microbiological risk assessment, shiga toxin producing *Escherichia coli* (STEC) and food attribution, characterization and monitoring, chapter 4-hazard identification and characterization, series 31.

Forsberg KJ, Reyes A, Wang B, selleck EM, Sommer MO, Dantas G (2012) The shared antibiotic resistance of soil bacteria and human pathogens. Science 337:1107-1111.

Foster G, Hopkins GF, Gunn GJ, Ternent HE, Thomson-Carter F, Knight HI, Graham DJ, Edge V, Synge BA (2003) Comparison of two pre-enrichment media prior to immunomagnetic separation for isolation of *Escherichia coli* O157 from bovine feces. J Appl Microbiol 95:2513-2516.

Franz E, Klerks MM, Devos OJ, Termorshuizen AJ, van-Bruggen AHC (2007) Prevalence of shiga toxin-producing *Escherichia coli* stx1, stx2, eaeA, and rfbE genes and survival of E.coli O157:H7 in Manure from organic and low- Input convention in Dairy farms. Appl Environ Microbiol 73:2180-2190.

Garbaj AM, Awad EM, Azwai SM, Abolghait SK, Naas HT, Moawad AA, Gammoui FT, Barbieri I, El-daghayes IM (2016) Enterohemorrhagic *Escherichia coli* O157 in milk and dairy products from Libya: Isolation and molecular identification by partial sequence of 16S rDNA. Vet World J 9:1184-1189.

Garcia PG, Silva VL, Diniz CG (2011) Occurrence and antimicrobial drug susceptibility patterns of commensal and diarrhea genic *Escherichia coli* in fecal micro biota from children with and without acute diarrhea. J Microbiol 49:46-52.

Hassan GM, Meshref AMS, El-Newery HA, Mohamed AS (2021) Prevalence of *Escherichia coli* in milk and some Dairy products in Beni-Suef Governorate, Egypt. J Vet Med Res 27:161-167.

Hassan MNA, Mehriz AM, Salem AS, Abozied HH (2017) Formulation and characterization aspects of light sour cream. J Food and Dairy Sci Mansoura Univ. 8:257-262.

Heijnen L, Medema G (2006) Quantitative detection of *E. coli*, *E. coli* O157 and other shiga toxin producing *E. coli* in water samples using a culture method combined with real-time PCR. J Water Health 4:487-498.

Ibrahim EMA, Sobeih AM (2006) Diarrhogenic E.coli in Karish cheese manufactured by different methods with special reference to E. coli O157:H7. Minfya Vetmed J 4:169-178.

Jiang J, Larkin C, steele M, Poppe C, Odumeru JA (1998) Evaluation of universal pre enrichment broth for the recovery of foodborne pathogens from milk and cheese. J Dairy Sci 81:2798-2803.

Kok T, Worswich D, Gowans E (1996) Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee J, Fraser A, Marmion B, Simmons A, eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.

Law D (2000) Virulence factors of *Escherichia coli* O157 and other shiga toxin producing E.coli. J Appl Microbiol 88:729-745.

Leininger DI, Roberson IR, Elvinger F (2001) Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram negative mastitis pathogens. J Vet Diagn Invest 13:273-275.

Losada L, DebRoy C, Radune D, Kim M, Sanka R, Brinka CL, kanyawasam S, Shelton D, Fratamico PM, Kapur V, Feng PCH (2016) Whole genome sequencing of diverse shiga toxin producing and nonproducing *Escherichia coli* strains reveals a variety of virulence and novel antibiotic resistance plasmids. Microorganisms J 8:8-11.

Mahanti A, Samanta I, Bandopaddhay S, Joardar SM, Dutta TK, Batahyal S, Sar TK, Isore DP (2013) Isolation, molecular characterization and antibiotic resistance of shiga toxin producing *Escherichia coli* (STEC) from buffalo in India. Lett Appl Microbiol 56:291-298.

March SB, Ratnam S (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 23:869-872.

Mir RA, Kudva IT (2019) Antibiotic resistant shiga toxin producing *Es-*

cherichia coli: An over view of prevalence and intervention strategies. Zoonoses Public Health 66:1-13.
Munita JM, Arias CA (2016) Mechanisms of Antibiotic resistance. Microbiol. Spectr 4:10.1128/microbiolspec.VMBF-0016-2015.
Pennington H (2010). *Escherichia coli* O157. Lancet 376:1428-1435.
Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153-2162.
Prencipe V, Migliorati G, Matteucci O, Calistri P, Di Giannatale E (2010) Assessment of hygienic quality of some types of cheese sampled from retail outlets. Vet Ital 46:233-242.
Trepeta RW, Edberg SC (1984) Methylumbelliferyl-D-Glucuronide-Based Medium for Rapid Isolation and Identification of *Escherichia coli*. J Clin Microbiol 19:172-174.
Salguero JF, AlCala M, Marcos A, Esteban M (1986) Measurement and calculation of water activity in blue cheese. J Dairy Res 52:639-644.
Sambrook J, Fritscgh EF, Mentiates (1989) Molecular coloning. A laboratory manual. Cold Spring Harbor Laboratory Press, New York.