Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Occurrence of multidrug resistant shiga-toxigenic *E. coli* in retailed cheese in Zagazig city, Egypt

Samah S. Abd Ellatif[1], Seham Elbadry[2], Hend Saeed Nada[3], Lamiaa M. Reda[4], Asmaa Badr Moustafa Badr Tahoun[1*]

[1]Department of Food Control, Faculty of Veterinary Medicine, Zagazig University, Zagazig City, 44511, Sharkia Governorate, Egypt,
[2]Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University Zagazig City, 44511, Sharkia, Governorate, Egypt,
[3]Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Sharkia Governorate, Zagazig City, 44511, Egypt,
[4]Central Laboratory, Faculty of Veterinary Medicine, Zagazig University Zagazig City, 44511, Sharkia Governorate, Egypt

ARTICLE INFO

Keywords

E. coli
Cheese
Antimicrobial sensitivity testing;
Shiga toxins

Received 02/05/2021
Accepted 19/05/2021
Available On-Line 01/07/2021

ABSTRACT

Cheese is regarded as an essential dairy product that cover part of the human needs with vitamins and minerals. However, cheese is also implicated in many food poisonings outbreaks worldwide. This study conducted to estimate coliforms and *E. coli,* and to investigate the occurrence of shigatoxigenic *E. coli* in four cheese types mostly consumed in Zagazig city, Egypt. In addition, detection of coding genes for shiga toxins (*stx1*, and *stx2*) was further screened using PCR. Besides, antimicrobial susceptibility of the isolated *E. coli* was further examined. The obtained results revealed that Kariesh cheese had the highest coliforms and *E. coli* counts, followed by Rumy, Domiati, and Feta cheeses, respectively. The isolation rates of *E. coli* were 52%, 24%, 20%, and 8% in Kariesh, Rumy, Domiati and Feta cheeses, respectively. The identified *E. coli* serotypes were *E. coli O55:H7, E. coli O86:H11, E. coli O78:H-, E. coli O111:H4, E. coli O127:H6,* and *E. coli O26:H11*. The expression of shiga toxin-coding genes indicated that *E. coli O55:H7* harbored only *stx1*, *E. coli O111:H4,* and *E. coli O86:*H11 harbored only *stx2, E. coli O78:H-,* and *E. coli O26:*H11 harbored both *stx1*, and *stx2*; however, *E. coli O127:H6* did not express any of the tested genes. Antimicrobial sensitivity testing revealed multidrug resistance profiles, particularly among *E. coli O86:*H11*, E. coli O78:H-,* and *E. coli O26:*H11. Therefore, strict hygienic measures should be adopted during all manufacture steps of these kinds of cheese.

1. INTRODUCTION

Cheese is rich in the essential amino acids, trace elements and minerals such as calcium and magnesium, and vitamins (Gerosa and Skoet, 2013; Ma et al., 2020). In Egypt there are many cheese types that are made from either raw or pasteurized milk such as Kariesh, Feta, Domiati, and Rumy cheeses. Cheese consumption is associated with many food poisonings outbreaks worldwide. One of the major organisms responsible for such food poisoning outbreaks is *Escherichia coli (E. coli)* (Mc Sweeney, 2007).

E. coli is a normal inhabitant in the intestinal flora of the farm animals. Detection of *E. coli* in foods of animal origin indicates fecal contamination. At the same time, *E. coli* is associated with several cases of human hospitalization (Darwish et al., 2015). *E. coli* strains are broadly classified into enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and shiga toxin-producing *E. coli* (STEC). This classification is dependent on their pathogenicity and virulence attributes (Ruttler et al., 2006; Xia et al., 2010). There are more than 100 *E. coli* serotypes that are classified as STEC. Of these, *E. coli* O157 causes 50 to 90% of *E. coli*-related human food poisoning cases, with most of the remaining cases caused by O45, O26, O111, O103, O121 and O145 (Scallan et al., 2011).

The uncontrolled usage of the antimicrobials in the animal production units had resulted in the development of antimicrobial resistance among microorganisms making treatment of infectious diseases as a challenging problem (Darwish et al., 2013). The present study was conducted to estimate the coliforms and *E. coli* counts and to examine the isolation rates of different *E. coli* serotypes in four cheese types (Kariesh, Feta, Domiati, and Rumy) mostly consumed in Zagazig city, Egypt. In addition, detection of shiga toxin-coding genes (*stx1*, and *stx2*) was done using PCR. Besides, antimicrobial sensitivity testing of the identified serotypes was further examined using the disk diffusion method.

2. MATERIAL AND METHODS

2.1. Sample collection:

One hundred cheese samples of Kariesh, Feta, Domiati, and Rumy, (n = 25/each cheese type) were collected from different

* Corresponding author: Asmaa B.M.B. Tahoun, E-mail address: abbadr@vet.zu.edu. dr.asmaatahoun@gmail.com.

grocery stores and street vendors in Zagazig city, Egypt. The microbiological examination of cheese samples was done at Faculty of Veterinary Medicine, Zagazig University.

2.2. Sample preparation:
Twenty-five grams from each sample was blended aseptically in buffered peptone water 0.1% (225 ml) for 2 min at 2500 rpm to obtain a dilution of 10^{-1}, followed by making decimal serial dilutions (APHA, 2001).

2.3. Estimation of MPN of coliforms:
The three tubes method recommended by APHA (2001) for the estimation of MPN of coliforms was followed.

2.4. Estimation of MPN of E. coli:
Loopfuls from the positive tubes (with acid and gas production) were aseptically inoculated into previously warmed tubes (44.5°C) containing 7 ml of *E. coli* (EC) broth (Himedia, Mumbai) and then incubated at 44.5°C for 24-48 hrs (APHA, 2001). The positive tubes were used to estimate MPN of *E. coli* according to the recommended tables.

2.5. Isolation of Escherichia coli:
From each positive tube (acid and gas) of EC broth, a loopful was streaked onto Eosin Methylene blue (EMB) agar plates, followed by incubation at 37°C for 24 hrs. Typical colonies of *E. coli* appear greenish, metallic with dark purple center. *E. coli* identification was done according to the staining and biochemical tests (APHA, 2001).

2.6. Sero-diagnosis of E. coli:
The rapid diagnostic *E. coli* antisera sets purchased from Difco, Detroit, USA were used for the serological identification of the confirmed *E. coli* isolates (Kok et al., 1996).

2.7. DNA preparation:
DNA was extracted from the confirmed *E. coli* isolates using the method reported before (Darwish et al, 2015).

2.8. Detection of shiga toxin-coding genes in the identified isolates:
Further detection of the coding genes for shiga toxins (*stx1* and *stx2*) in the identified *E. coli* isolates was carried out using multiplex PCR. The primer sequences and the amplified products sizes were presented in Table 1. The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Germany). PCR assays were carried out using the method of Dhanashree and Mallya (2008) using AmpliTaq DNA polymerase kit (Perkinelmer). *E. coli* O157:H7 Sakai was used as positive strain for *stx1,* and *stx2.* While *E. coli* K12DH5α was used as a negative control. Visualization of the DNA fragments was done using agarose gel electrophoresis 2% in 1x TBE buffer. A 100 bp plus DNA Ladder was used as the experimental marker.

Table 1. Oligonucleotides' sequences of shiga toxin-coding genes

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	Dhanashree and Mallya (2008)
stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3	779	
stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		

2.9. Antimicrobial sensitivity testing of E. coli:

Antimicrobial sensitivity testing was conducted using the disk diffusion method (Wayne, 2013). The tested antimicrobials were ampicillin (AM) 10 µg, cephalothin (CN) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CP) 5 µg, enrofloxacin (En) 5 µg, erythromycin (E) 15 µg, gentamicin (G) 10 µg, kanamycin (K) 30 µg, nalidixic acid (NA) 30 µg, neomycin (N) 30 µg, oxacillin (OX) 1 µg, oxytetracycline (T) 30 µg, penicillin (P) 10 IU and trimethoprim/sulfamethoxazole (SXT) 25 µg.

2.10. Statistical analysis
Coliforms and *E. coli* counts were expressed as means ± SE for log 10 cfu/g. Statistical analysis was done using Tukey–Kramer HSD test where, p <0.05 indicated statistical differences (Gomez and Gomez, 1984).

3. RESULTS

Figure 1A showed that Kariesh cheese had the highest coliforms counts (log 10 cfu/g) among the examined cheese samples with a value of 4.15 ± 0.15, followed by Rumy cheese (3.87 ± 0.17), Domiati cheese (3.45 ± 0.21), and Feta cheese (2.45 ± 0.11), respectively. *E. coli* counts (log 10 cfu/g) in the examined cheese samples were corresponding to the coliforms counts as Kariesh cheese had significantly the highest value (3.85 ± 0.18), followed by Rumy cheese (3.17 ± 0.12), Domiati cheese (2.75 ± 0.19), and Feta cheese (2.11 ± 0.22), respectively (Fig. 1B).
The prevalence rates of *E. coli* were 52%, 24%, 20%, and 8% in Kariesh, Rumy, Domiati and Feta cheeses, respectively (Fig. 2). The isolated *E. coli* were further identified into six serotypes namely *E. coli O55:H7, E. coli O111:H4, E. coli O127:H6, E. coli O86:H11, E. coli O78:H,* and *E. coli O26:H11*. Kariesh cheese had the highest contamination level with different *E. coli* serotypes, particularly with *E. coli O78:H-* (20%), and *E. coli O26:H11* (16%), while Feta cheese had the lowest contamination level (Fig. 3A, B).

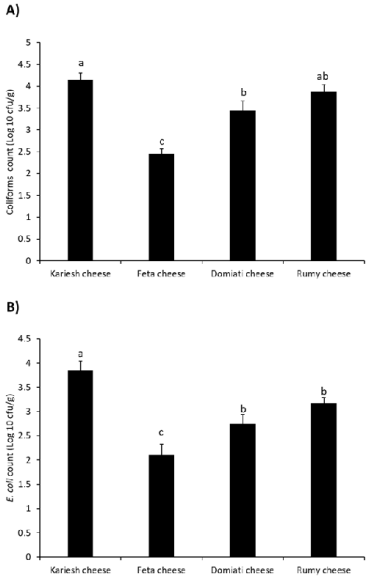

Figure 1. Coliforms and *E. coli* counts in the examined cheese samples. Values represent means ± SE (Log 10 cfu/g) of A) Coliforms B) *E. coli counts* in Kariesh, Feta, Domiati, and Rumy cheeses. Columns with different letter are significantly different at $p < 0.05$.

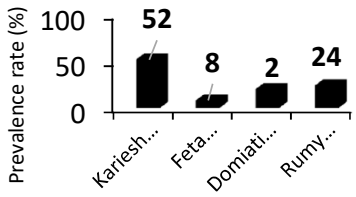

Figure 2. Prevalence of *E. coli* in examined cheese samples.
Prevalence rates (%) of *E. coli* in Kariesh, Feta, Domiati, and Rumy cheeses (n=25 each)

We further detected the expression of shiga toxin-coding genes among the identified *E. coli* serotypes. *E. coli O55:H7* harbored only *stx1*, *E. coli O111:H4,* and *E. coli O86:*H11 harbored only *stx2, E. coli O78:H-,* and *E. coli O26:*H11 harbored both *stx1*, and *stx2*; however, *E. coli O127:H6* did not express any of the tested genes (Fig. 4).
Antimicrobial sensitivity testing revealed drug resistance for more than one antimicrobial, particularly in *E. coli O86:*H11*, E. coli O78:H-,* and *E. coli O26:*H11 (Table 2).

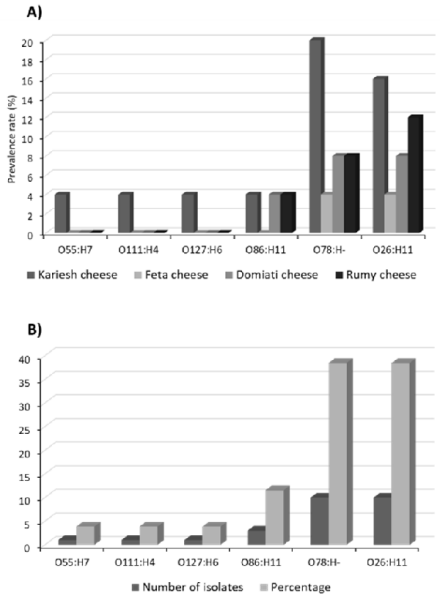

Figure 3. Prevalence rates of different *E. coli* serotypes A) in examined cheese types; B) Number and percentages of each serotype

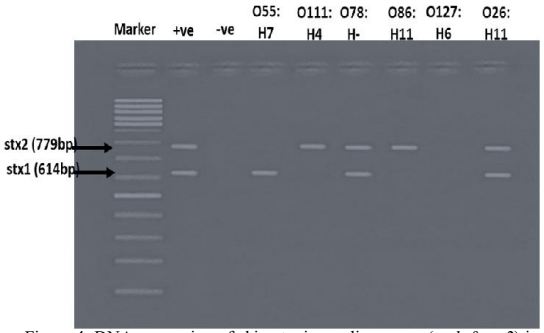

Figure 4. DNA expression of shiga toxins coding genes (*stx1 & stx2*) in the identified *E. coli* serotypes isolated from examined retailed cheese samples.

Table 2: Antimicrobial sensitivity testing of *E. coli* serotypes identified in the examined cheese samples.

	O55:H7		O111:H4		O127:H6		O86:H11		O78:H-		O26:H11	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AM	1	100	1	100	1	100	3	100	7	70	6	60
CN	0	0	1	100	1	100	0	0	3	30	3	30
C	0	0	0	0	1	100	1	33.33	3	30	2	20
CP	0	0	0	0	1	100	1	33.33	4	40	2	20
En	0	0	0	0	1	100	0	0	2	20	4	40
E	0	0	0	0	0	0	1	33.33	4	40	4	40
G	0	0	0	0	0	0	2	66.66	1	10	1	10
K	0	0	0	0	0	0	1	33.33	1	10	2	20
NA	1	100	1	100	1	100	3	100	8	80	6	60
N	0	0	0	0	0	0	0	0	1	10	1	10
OX	0	0	0	0	1	100	2	66.66	4	40	5	50
T	0	0	0	0	0	0	1	33.33	4	40	4	40
P	1	100	1	100	1	100	3	100	10	100	10	100
SXT	0	0	1	100	1	100	2	66.66	5	50	3	30

No.: Number of isolates
%: Percentage of isolates
AM: Ampicillin; CN: Cephalothin; C: Chloramphenicol; CP: Ciprofloxacin; En: Enrofloxacin; E: Erythromycin; G: Gentamicin; K: Kanamycin; NA: nalidixic acid; N: Neomycin; OX: Oxacillin; T: Oxytetracycline; P: Penicillin; SXT: trimethoprim/sulfamethoxazole

4. DISCUSSION

In the present study, Kariesh cheese had the highest coliforms counts as well as*, E. coli* counts among the examined cheese samples followed by Rumy, Domiati then Feta cheeses, respectively. Such high values of coliforms and *E. coli* counts reflect improper hygienic measures adopted during cheese preparation, storage, or distribution, particularly in the case of Kariesh cheese (Mossel et al., 1995). Similarly, unsatisfactory hygienic measures were reported for the retailed fresh cheese in Mexico (de la Rosa-Hernández et al., 2018), and in Kariesh cheese, Ras cheese, and Tallaga cheese retailed in Beni-Suef city, Egypt (Hassan et al., 2019).
E. coli is considered as a major foodborne pathogen responsible for many cases of hospitalization and deaths especially among children and elderly. For instance, a cluster of *E. coli O157:H7* hemorrhagic colitis was identified in Canada, between October 2002 and February 2003 (Honish et al., 2005). Besides, *E. coli O104:H4* was responsible for an outbreak occurred in Germany in May 2011 infecting more than 3000 peoples and left 50 deaths (Frank et al., 2011). In addition, another 19 persons were infected with the shiga toxin producing *E. coli O121* in six of United States (CDC 2014). In the present study, the highest prevalence rate of *E. coli* was in Kariesh cheese followed by Rumy, Domiati and Feta cheeses, respectively. In agreement with the obtained results in the present study, Hassan and Elmalt (2008) isolated toxigenic *E. coli* from retailed Kariesh cheese in Qena city at 47.8%. Besides, Ombarak et al. (2016) isolated *E. coli* of enteropathogenic and enterohemorrhagic types at 74.5% from Kariesh cheese, and 21.7% from Ras cheese retailed in Egypt. Furthermore, Hussein et al. (2019) isolated *E. coli* at 16%, and 5.3% from Kariesh cheese and Ras cheese, respectively, sold in Menoufia Governorate.
E. coli O55:H7, E. coli O111:H4, E. coli O127:H6, E. coli O86:H11, E. coli O78:H-, and *E. coli O26:H11* were further identified as the prevalent serotypes in the current investigation. Kariesh cheese had the highest contamination level with different *E. coli* serotypes, particularly with *E. coli O78:H-* and *E. coli O26:H11*, while Feta cheese had the lowest contamination level. Similarly, de Campos et al. (2018) could identify *E. coli O127, O73:H12,* and *O64474:H8* from Minas cheese in Brazil. Furthermore, Hussein et al. (2019) identified eight *E. coli* serotypes from Kariesh and Ras cheeses, namely, *E. coli O26: H11, O91: H21, O111: H2, O103: H2, O125: H21, O171: H2, O86:H, and O119: H6.*

The expression of shiga toxin-coding genes among the identified *E. coli* serotypes were detected. The obtained results revealed that *E. coli O55:H7* harbored only *stx1*, *E. coli O111:H4,* and *E. coli O86:*H11 harbored only *stx2, E. coli O78:H-,* and *E. coli O26:*H11 harbored both *stx1*, and *stx2*; however, *E. coli O127:H6* did not express any of the tested genes. *E. coli* of non-O157 serogroups such as *O26, O103, O111* were reported to be the most substantial food poisoning pathogen groups, especially *O26* that able to cause wide range of illness in human (Dambrosio et al., 2007). Similarly, *E. coli* expressing shiga toxin coding genes were isolated from a Spanish raw ewe's milk cheese (Caro et al., 2007). In addition, Elhadidy and Mohammed (2013) isolated shiga toxin-producing *E. coli* including serotypes *O22:H8, O26:H11, O86:H21, O103:H2, O113:H21* and *O146:H21* from Kariesh and Domiati cheese retailed in Egypt. Hussein et al. (2019) could also identify eight *E. coli* serotypes from Kariesh and Ras cheeses producing shiga toxins (*stx1, and stx2*). STEC is implicated in many cases of hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Karch et al., 2005).

The uncontrolled usage of antimicrobials in the livestock production is the major cause for the development of drug-resistance pathogens, which is regarded as a major health concern. In the current study, the multidrug resistance profiles particularly among *E. coli O86:*H11*, E. coli O78:H-*, and *E. coli O26:*H11 were prominent. For instances, 50% or more of the identified *E. coli O26:H11* showed resistance to AM, P, NA, and OX. While 50% or more of *E. coli O86:*H11 showed resistance to AM, G, NA, P, OX, and SXT. Isolation of multidrug resistant *E. coli* from cheese was reported in studies conducted in Romania (Tabaran et al., 2017), Brazil (de Campos et al., 2018), and Egypt (Ombarak et al., 2018). Therefore, rational use of antimicrobials in the animal farms and livestock production is highly recommended.

In conclusion, the current study revealed isolation and identification of multidrug resistant and shiga toxin-producing *E. coli* from Kariesh, Domiati, Rumy and Feta cheese retailed in Zagazig city, Egypt. Therefore, strict observation of hygienic measures should be adopted during all manufacture steps of these kinds of cheese. In addition, continuous monitoring studies for the prevalence of STEC in other dairy products are highly recommended.

REFERENCES

1. APHA 'American Public Health Association ', 2001. Compendium of methods for the microbiological examination of food, 4th Ed. American Public Health Association, Washington, D.C.

2. Caro, I., García-Armesto, M.R., 2007. Occurrence of Shiga toxin-producing Escherichia coli in a Spanish raw ewe's milk cheese. International Journal of Food Microbiology 116(3):410-3.
doi: 10.1016/j.ijfoodmicro.2007.02.015.

3. Centers for Disease Control and Prevention (CDC), 2014b. Multistate Outbreak of Shiga toxin-producing *Escherichia coli* O121 Infections linked to raw Clover Sprouts (Final Update).
http://www.cdc.gov/ecoli/2014/O121-05-14/index.html.

4. Dambrosio, A., Lorusso, V., Quaglia, N.C., Parisi, A., La Salandra, G., Virgilio, S., Mula, G., Lucifora, G., Celano, G.V. Normanno, G., 2007. Escherichia coli O26 in minced beef: prevalence, characterization and antimicrobial resistance pattern. International Journal of Food Microbiology 118(2): 218-222.

5. Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S., Ishizuka, M., 2013. Antibiotic residues in food: The African scenario. Japanese journal veterinary research 61 Suppl:S13-22.

6. Darwish, W.S., Saad Eldin, W.F., Eldesoky, K.I., 2015. Prevalence, molecular characterization and antibiotic susceptibility of Escherichia coli isolated from duck meat and giblets. Journal of Food Safety 35: 410-415.

7. de Campos, A.C.L.P., Puño-Sarmiento, J.J., Medeiros, L.P., Gazal, L.E.S., Maluta, R.P., Navarro, A., Kobayashi, R.K.T., Fagan, E.P., Nakazato, G., 2018. Virulence Genes and Antimicrobial Resistance in Escherichia coli from Cheese Made from Unpasteurized Milk in Brazil. Foodborne Pathogen Disease 15(2):94-100.
doi: 10.1089/fpd.2017.2345.

8. de la Rosa-Hernández, M.C., Cadena-Ramírez, A., Téllez-Jurado, A., Gómez-Aldapa, C.A., Rangel-Vargas, E., Chávez-Urbiola, E.A., Castro-Rosas J., 2018. Presence of Multidrug-Resistant Shiga Toxin-Producing *Escherichia coli*, Enteropathogenic Escherichia coli, and Enterotoxigenic *Escherichia coli* on Fresh Cheeses from Local Retail Markets in Mexico. Journal of Food Protection 81(11):1748-1754. doi: 10.4315/0362-028X.JFP-18-166.

9. Dhanashree, B., Mallya, S., 2008. Detection of shiga-toxigenic *Escherichia coli* (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Indian Journal of Medical Research 128: 271-277.

10. Elhadidy, M., Mohammed, M.A. 2013. Shiga toxin-producing Escherichia coli from raw milk cheese in Egypt: prevalence, molecular characterization and survival to stress conditions. Letters in Applied Microbiology 56(2):120-7. doi: 10.1111/lam.12023.

11. Frank, C.D., Werber, J.P., Cramer, M., Askar, M., Faber, M., Ander Heiden, H., Bernard, A., Fruth, R., Prager, A., Spode, M., Wadl, A., Zoufaly, S., Jordan, M.J., Kemper, P., Follin, L., Muller, L.A., King, B., Rosner, U., Buchholz, K., Krause, G., 2011. Epidemic profile of Shiga-toxin-producing Escherichia coli O104: H4 outbreak in Germany. New England Journal of Medicine 365: 1771-1780.

12. Gerosa, S., Skoet, J., 2013. Milk availability: Current production and demand and medium-term outlook, Chapter 2. In: Muehlhoff E. BA, McMahon D (Editor), Milk and dairy products in human nutrition. Food and Agriculture Organization of the United Nations, Rome, pp. 11-40.

13. Gomez, K.A., Gomez, A.A. 1984. Statistical procedures for agriculture research. John Wiliy and Sons Editor Inc. USA (2Ed.), Chapter 3:129-184.

14. Hassan, S.A., Elmalt, L.M., 2008. Informally raw milk and Kareish cheese investigation on the occurrence of toxigenic Escherichia coli in Qena City, Egypt with emphasis on molecular characterization. Assiut University Bulletin for Environmental Researches 11(2):35-42.

15. Hassan, G.M., Meshref, M.S.A., Zeinhom, M.A.M., Abdel-Halem, M.S., 2019. Impact of spoilage microorganisms on some dairy products. Assiut Veterinary Medical Journal 65(161):133-141.

16. Hussien, H., Elbehiry, A., Saad, M., Hadad, G., Moussa, I., Dawoud, T., Mubarak, A., Marzouk, E., 2019. Molecular characterization of *Escherichia coli* isolated from cheese and biocontrol of Shiga toxigenic E. coli with essential oils. Italian Journal of Food Safety 8(3):8291. doi: 10.4081/ijfs.2019.8291.

17. Karch, H., Tarr P.I., Bielaszewska, M., 2005. *Enterohaemorrhagic Escherichia coli in human medicine.* International Journal of Medical Microbiology 295(6-7): p. 405-418.

18. 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. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.
19. Ma, J.K., Raslan, A.A., Elbadry, S., El-Ghareeb, W.R., Mulla, Z.S., Bin-Jumah, M., Abdel-Daim, M.M., Darwish, W.S., 2020. Levels of biogenic amines in cheese: correlation to microbial status, dietary intakes, and their health risk assessment. environmental science and pollution research international 27(35):44452-44459. doi: 10.1007/s11356-020-10401-2.
20. McSweeney, P.L.H., 2007. Pathogens and food poisoning bacteria, In Woodhead Publishing Series in Food Science, Technology and Nutrition, Cheese Problems Solved, Woodhead Publishing, Pages 133-151, https://doi.org/10.1533/9781845693534.133.
21. Mossel, D.A.A., Corry, J.E.L., Struijk, C.B., Baird, R.M., 1995. Essentials of the microbiology of foods: a textbook for advanced studies. Chichester (England): John Wiley & Sons. 287-289.
22. Ombarak, R.A., Hinenoya, A., Awasthi, S.P., Iguchi, A., Shima, A., Elbagory, A.M., Yamasaki, S., 2016. Prevalence and pathogenic potential of Escherichia coli isolates from raw milk and raw milk cheese in Egypt. International Journal of Food Microbiology 221:69-76.
doi: 10.1016/j.ijfoodmicro.2016.01.009.
23. Ruttler, M.E., Yanzon, C.S., Cuitino, M.J., Renna, N.F., Pizarro, M.A., Ortiz, A.M., 2006. Evaluation of a multiplex PCR method to detect enteroaggregative Escherichia coli. Biocell 30: 301-8.
24. Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States - Major pathogens. Emerging Infectious Diseases journal 17(1):7-11.
25. Tabaran, A., Mihaiu, M., Tăbăran, F., Colobatiu, L., Reget, O., Borzan, M.M., Dan, S.D., 2017. First study on characterization of virulence and antibiotic resistance genes in verotoxigenic and enterotoxigenic E. coli isolated from raw milk and unpasteurized traditional cheeses in Romania. Folia Microbiologica (Praha) 62(2):145-150. doi: 10.1007/s12223-016-0481-8.
26. Wayne P., 2013. Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100–S23. Clinical and Laboratory Standards Institute 33, 118-156.
27. Xia, X., Meng, J., Mcdermott, P.F., Ayers, S., Blickenstaff, K., Tran, T.T., Abbott, J., Zheng, J., Zhao, S., 2010. Presence and characterization of shiga toxin-producing Escherichia coli and other potentially diarrheagenic E. coli strains in retail meats. Applied and Environmental Microbiology 76: 1709-17.