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Occurrence of Virulent and Antibiotic-resistant Enteropathogenic and Shiga toxin- producing *Escherichia coli* in some Milk Products Sold in Assiut City, Egypt

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ABSTRACT

This study was undertaken to detect the enteropathogenic and shiga toxin-producing *Escherichia coli* (EPEC and STEC) in 120 milk products samples (soft cheese, hard cheese, yoghurt and ice cream). All samples were submitted for bacteriological examination, serological and molecular identification of virulent and antibiotic resistant genes using *eaeA*, *hylA*, bla_{TEM}, bla_{CTX-M1}, bla_{OXA} and bla_{SHV} primers. The bacteriological examination revealed that the incidence of occurrence of EPEC was 3.33% in ice cream samples, while it could not isolated from other types of milk products. In addition, STEC failed to detect in all examined milk products. The isolated EPEC strain following *E. coli* O18 serotyping. Moreover, the molecular identification of the isolated strain revealed that the strain contains *eaeA*, bla_{TEM} and bla_{CTX-M1} genes.

Introduction

Milk products are considered the most popular Egyptian food and play a prominent role in the Egyptian diet. The manufacture of these products is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products. Beside their manufacture, the handling techniques in Egyptian markets are still primitive and unhygienic.

Many enteropathogenic microorganisms have been found in milk products specially that not subject to efficient heat treatment. They are frequently associated with several outbreak (Almeida Filho and Nader Filho, 2002). One of these bacteria is *Escherichia coli*, which is normally inhabit in intestine of animal and human (Wetzel, 2005). Pathogenic strain of *E. coli* can be divided according to the mechanism of action into diarrhoeagenic *E. coli* and extra intestinal *E. coli* (Croxall *et al*., 2011) based on the site of infection. *E. coli* strain harbor various virulence factors including enteropathogenic *E. coli*, enterotoxigenic *E. coli* (ETEC), enterohemorrhagic/ Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Kaper *et al*., 2004) which lead to gastrointestinal tract disease, including watery or bloody diarrhea and might develop life-threating disease. While, extra intestinal *E. coli* infection causes many diseases as uropathogenic *E. coli* (UPEC) causing urinary tract infection, *E. coli* strains that cause septic anemia, and neonatal meningitis associated *E. coli* (MNEC) (Bekal *et al*., 2003).

Many virulence factors responsible for previous diseases as two phage encoded cytotoxins (stx_1 and stx_2) (Kawano *et al*., 2012), intimin (*eaeA*) and hemolysin (*hlyA*) (Slanec *et al*., 2009). The most prevalent serotypes with the enteropathogenic *E. coli* (EPEC) are O18, O20, O25, O26, O44, O55, O86, O91, O111, O114, O119, O125 ac, O126, O127, O128, O14 and O158 (Nataro and Kaper, 1998) these serotypes linked epidemiologically to infantile diarrhea. While, the most prevalent serotypes associated with (STEC) human infections are O157, O111, O26, O103, O113, O91, O118, O121, O145, O128 and O146 (Rahal *et al*., 2015).

The treatment of gastroenteritis caused by *E. coli* requires antimicrobial therapy. However, currently there has been a rise in the occurrence of antibiotic-resistant *E. coli* due to the presence of resistance genes in *E. coli*. These genes responsible for production of hydrolysis enzymes, which lead to destruction

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of the beta-lactamase ring of the antibiotics. These enzymes known as extended spectrum β-lactamases (ESBLs) such as TEM, CTX, OXA and SHV enzymes (Bush and Jacoby, 2010).

Considering the above facts the present study was designed to determine the occurrence of virulence and antibiotic-resistant genes profile of enteropathogenic *E. coli* isolated from some milk products sold in Assiut City, Egypt.

Materials and methods

Sample and study area

A total of 120 random samples of some milk products including: soft cheeses (Kareish and Damietta), hard cheeses (Cheddar and Ras), yoghurt and ice cream (30 for each) were been collected from different places in Assiut City in clean, dry and sterile containers, while ice cream samples were taken in the ice boxes. These samples were transferred to the laboratory as soon as possible to be examined.

Preparation of samples

Twenty five grams from each sample were thoroughly mixed before being examined according to A.P.H.A. (2004).

Isolation of EPEC and STEC

Enrichment procedure (Samadpour *et al*., 1990)

A tube of modified vancomycin-trypticase soya broth (mvTSB) was inoculated with 1 ml prepared sample. Mixed well and incubated at 37 ºC for 24.0 ± 2.0 hrs.

Plating on selective agar media (A.P.H.A., 2004)

A loopful of incubated broth was subcultured into the surface of Sorbitol MacConkey (SMAC) agar followed by incubation at 37°C for 24 hrs.

Identification of EPEC

Biochemical tests

Triple Sugar Iron (TSI), Indol test, Methyl Red Test, Voges Proskaur test and Citrate utilization test were used as biochemical tests to identify the isolated strains (A.P.H.A., 2004).

Serological tests

The identified *E. coli* isolate was serotyped by slide agglutination test in the serological laboratory of Animal Health Research Institute (Cairo, Egypt) using standard polyvalent and monovalent EC antisera according to Hamed *et al*. (2017).

Polymerase chain reaction (PCR)

DNA extraction

The suspected bacterial colony was subcultured onto TSB and incubated overnight at 37°C for DNA extraction using Qiagen DNA blood Mini kit (Cat.No. 51104, Hilden, Germany) according to manufacturer instruction. The extracted DNA was stored at -20°C.

DNA amplification was performed in final volume 25 µl, which contained the following,12.5 µl of 2X PCR master mix (Green Master, Promega, USA), 150 ng of the DNA template, 0.5 µM of each primer (Table 1) and up to 25 µl Nuclease free water, the mixture was mixed in a PCR tube. The virulent factors genes amplification was performed in a programmable gradient thermal cycler (Veriti Applied Biosystem, USA) at 94°C for five min, followed by 35 cycles were run under the following conditions; denaturation at 94°C for 50 sec, annealing at 63°C for 50 sec and extension at 72°C for 1 min. At the end, the preparations were kept for 10 min at 72°C as final extension. While, the thermal profile of antibiotic-resistant genes amplification was at 95°C initial denaturation step for 5 min followed by 35 cycles of 95°C for 40 sec, 51°C for 40 sec, and 72°C for 90 sec. The final cycle was followed by 72°C incubation for 10 min.

Gel Electrophoresis

PCR products were electrophoresed in 1% agarose gel (GX 040.90, Gen AGarose, L.E., Standard DNA /RNA agarose, Molecular Biology Grade, Inno–Train Diagnostic, D–61476, Kronberg/Taunus) Containing Ethidium bromide as 1µl /ml electrophoresis buffer at 100 volts for 60 min. Using 100 bp DNA–ladder in (SCiE–PLAS, HU 10, 5636, UK). The result obtained through High performance ultraviolet Transilluminator,

Table 1. Primers used to amplify fragments of genes responsible for expression of different virulence factors and antibiotic-resistant genes for E. coli.

Primer Name	Sequence (5'-3')	Target gene	PCR product (bp)	Reference
AE22 AE20-2	ATTACCATCCACACAGACGGT ACAGCGTGGTTGGATCAACCT	*eaeA*	397	Sarimehmetoglu et al. (2009)
MFS1-F MFS1-R	ACGATGGTTTATTCTGGA CTTCACGTCATCACCATACATAT	*hly*	166	
BlaTEM –F BlaTEM -R	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	bla_{TEM}	800	Ogutu et al. (2015)
BlaCTX-M1-F BlaCTX-M1-R	TTAGGAAGTGTGCCGCTGTA CGGTTTTATCCCCCACAAC	bla_{CTX_M1}	655	
BlaOXA-F BlaOXA-R	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	bla_{OXA}	564	Perez et al. (2007)
BlaSHV-F BlaSHV-R	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	bla_{SHV}	713	

Table 2. Incidence of EPEC and STEC in the examined milk products samples

Examined samples	No. of examined samples	ECEC Positive samples		STEC Positive samples	
		No.	%	No.	%
Soft cheese	30	-	0	-	0
Hard cheese	30	-	0	-	0
Yoghurt	30	-	0	-	0
Ice cream	30	1	3.33	-	0

(UV, INC, UK). The image of the PCR products containing the positive DNA sequence of for the positive genes were documented using DOC–It ® LS, Image acquisition–software, (Biodoc Analyzer, Biometra, Germany).

Results

Results of this study revealed that EPEC could be detected only on one ice cream sample in percent 3.33%, while it could not be isolated from the soft cheese, hard cheese and yoghurt samples. Moreover, STEC didn't detect in the examined milk products (Table 2).

In addition, this study carried out the serotyping of isolated EPEC. The strain following *E. coli* O18 group with polyvalent 3, also, the strain contain *eaeA*, bla_{TEM} and blaCTX_M1 genes, on the other side, the strain didn't carry *hylA*, bla_{OXA} and bla_{SHV} as described in Table 3 and Fig. 1.

Table 3. Characterization of virulence factors and antibiotic-resistant of *E. coli* O18 by PCR.

Target Gene	Result	Target Gene	Result
eaeA		*hly*	
bla_{TEM}	Positive	bla_{OXA}	Negative
bla_{CTX_M1}		bla_{SHV}	

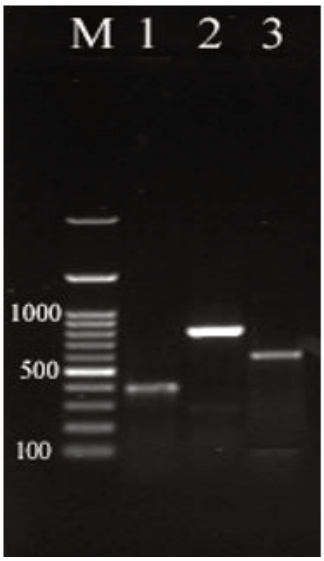

Fig. 1. PCR products of amplified of virulent and antibiotic resistant genes visualized on agarose gel electrophoresis. Lane (M) DNA ladder 100 bp, Lane (1) positive for gene *eaeA* at 397 bp, Lane (2) positive for gene bla_{TEM} at 800 bp, Lane (3) positive for gene bla_{CTX_M1} at 655 bp.

Discussion

Milk products have been associated with health benefits for many years containing bioactive peptide, probiotic bacteria, antioxidant, vitamins and highly absorbable calcium (Bhat and Bhat, 2011). However, the method of production, transportation, handling and sale of milk and milk products are entirely unhygienic which lead to contamination by food poisoning bacteria as *E. coli*.

The results of milk product samples revealed that the ice cream samples were contaminated with EPEC in percentage of 3.33. While, the others products (soft cheese, hard cheese and yoghurt samples) didn't contaminated by *E. coli*. By the serotyping the isolated strain was *E. coli* O18. The ice cream contaminated by *E. coli* O18 may be attributed to contamination of milk used in the manufacturing or due to post pasteurization and post processing contamination. On the other side, it didn't detect in the soft cheese, hard cheese and yoghurt samples due to the low pH of the previous products, which inhibit the growth of these bacteria. These result agree with Elbagory *et al.* (2015) result, they didn't isolate *E. coli* O18 from Kareish and Damietta cheese, while they isolate it from raw milk samples. Moreover, STEC as *E. coli* O157 failed to detect in the examined milk products. These results agree with that carried out by Ibrahim and Sobeih (2006) and Nazem *et al.* (2016), they also failed to detect *E. coli* O157:H7 from cheese samples.

E. coli O18 implicated in many outbreak as an outbreak of bloody diarrhea occurred in the neonatal nursery ward in Argentina, in 1996 (Chinen *et al.*, 2002). In addition, this strain is involved in a large range of extra intestinal infections in human as neonatal meningitis, septicemia, pyelonephritis and pneumonia (Russo and Johnson, 2000; Johnson and Russo, 2002; Mokady *et al.*, 2005; Ron, 2006), as well as, in animals such as mastitis in dairy animals (Lamey *et al.*, 2013).

Defining the virulence factors and mechanism of *E. coli* O18 pathogenesis has been on many literatures. The production of *eaeA* and *hylA* are considered essential but not solely responsible for disease. In our study, the isolate was carried *eaeA* gene, while, the isolated strain wasn't contain *hlyA* genes. The most important protein facilitate the pathogenicity of EPEC is intimin. Intimin is outer membrane protein encoded by *eaeA* gene which help the *E. coli* to intimate attachment to intestinal epithelial cells. The affected epithelial cells include phosphorylation, calcium influx and the rearrangement of cytoskeletal components under the adherent EPEC. So, it lead to reduce absorption capacity of the brush border and a stimulation of intestinal secretion (Wilshaw *et al.*, 2000).

The first pathotype of *E. coli* was the EPEC. This type was isolated from large outbreaks of infant in UK, in 1945 (Kaper *et al.*, 2004). The most lineament of EPEC infections is the attaching-effacing histopathology, which can be detected by intestinal biopsy specimens from patients or infected animals. In addition, EPEC infection is primarily a disease of infants

younger than 2 years (Nataro and Kaper, 1998). Beside the watery diarrhea, which is hallmark sign of EPCE, the vomiting and low-grade fever are common symptoms of it. Some studies in Brazil, Mexico, and South Africa have shown that 30-40% of infant diarrhea can caused by EPEC (Robins-Browne *et al*., 1980; Cravioto *et al*., 1990; Gomes *et al*., 1991).

E. coli has become the most important member for infection and colonization of food animals since the early 2000s (Aidara-Kane *et al*., 2013; Lahlaoui *et al*., 2014; Rao *et al*., 2014; Su *et al*., 2014). In this study, the isolated *E. coli* O18 strain contained two genes responsible for ESBL as bla_{TEM} and $bla_{CTX\text{-}M1}$, while the strain didn't have bla_{OXA} and bla_{SHV} genes. These result similar to the result obtained by Gundogan and Avci (2013), they detected only one strain of ESBL producing *E. coli* isolated from ice cream milk samples sold in Turkey. Many previous studies recorded the ESBL producing *E. coli* in raw milk and researchers concluded that the unclean hand worker, unhygienic conditions of milking, unclean water supplies used in washing the utensils, all the previous factors play an important role in contamination of the milk and milk products with antibiotic-resistant *E. coli*. In addition, the intensive use of antibiotics for animal production lead to increase the incidence of the antimicrobial resistance bacteria in milk and milk products (Tekiner and Özpınar, 2016; Badri *et al*., 2018 and Ombarak *et al*., 2018).

Conclusion

The results obtained from this study concluded that the milk products sold in the Assiut markets have EPEC contamination. Thus, the results of the study warn the need more strict preventive measure. So, certain recommendations should be followed as implementation of hygienic measures in dairy farms and dairy plants. Also, the present study suggest investigating the HACCP application to avoid milk products contamination by EPEC.

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References

A.P.H.A. (American Public Health Association), 2004. Standard Methods for the Examination of Dairy Products, 17th Ed., American Public Health Association, Washington, D.C.

Aidara-Kane, A., Andremont, A., Collignon, P., 2013. Antimicrobial resistance in the food chain and the AGISAR initiative. J. Infect. Public Health 6, 162–165.

Almeida Filho, E.S., Nader Filho, A., 2002. Ocorrência de coliformes fecais e *Escherichia coli* em queijo tipo minas Frescal de produção artesanal, comercializado em Poços de Caldas, MG. Higiene Alimentar 16, 71-73.

Badri, A.M., Ibrahim, I.T., Mohamed, S.G., Garbi, M.I., Ahmed S Kabbashi, A.S., Arbab, M.H., 2018. Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* and Klebsiella pneumoniae Isolated from Raw Milk Samples in Al Jazirah State, Sudan. Molecular Biology 7,1.

Bekal, S., Brousseau, R., Masson, L., Préfontaine, G., Fairbrother, J.M., Harel, J., 2003. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarray. Journal Clinical Microbiology 41, 2113–2125.

Bhat, Z.F., Bhat, H., 2011. Milk and dairy products as functional of foods: A Review. International Journal of Dairy Science 6(1), 1-12.

Bush, K., Jacoby, G.A., 2010. Updated functional classification of beta-lactamases. Antimicrobial Agents Chemotherapy 54, 969-976.

Chinen, I., Rivas, M., Soriano, V., Miliwebsky, E., Galvez, G.F., Chillemi, G., Baschkier, A., Wang, G., Caldeira, R., Woodward, D.L., Rodgers, F.G., 2002. *Escherichia coli* ehl1 Gene-Positive Serotype O18ac:H31 Associated with an Outbreak of Diarrhea in a Neonatal Nursery in Neuquén City, Argentina. Journal of Clinical Microbiology 40(40), 1225–1229.

Cravioto, A., Reyes, R.E., Trujillo, F., Uribe, F., Navarro, A., De La Roca, J.M., Hernandez, J.M., Perez, G., Vazquez, V., 1990. Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. American Journal of Epidemiology 131, 886–904.

Croxall, G., Hale, J., Weston, V., Manning, G., Cheetham, P., Achtman, M., McNally, A., 2011. Molecular epidemiology of extraintestinal pathogenic *Escherichia coli* isolates from a regional cohort of elderly patients highlights the prevalence of ST131 strains with increased antimicrobial resistance in both community and hospital care settings. Journal of Antimicrobial Chemotherapy 66, 2501–2508.

Elbagory, A.M., Eman, Sh. E., Eman, K.F., 2015. Impact of Probiotic Strains on Growth of Some Food Poisoning Bacteria from Milk and Soft Cheese. Nutrition Food Technology 1(2). doi http://dx.doi.org/10.16966/2470-6086.107

Gomes, T.A., Rassi, V., Mac Donald, K.L., 1991. Enteropathogens associated with acute diarrheal disease in urban infants in Sao Paulo, Brazil. Journal of Infectious Diseases 164, 331–337.

Gundogan, N., Avci, E., 2013. Prevalence and antibiotic resistance of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and Klebsiella species isolated from foods of animal origin in Turkey. African Journal of Microbiology Research 7(31), 4059-4064.

Hamed, O.M., Sabry, M.A., Hassanain, N.A., Eman Hamza, E., Hegazi, A.G., Salman, M.B., 2017. Occurrence of virulent and antibiotic-resistant Shiga toxin-producing *Escherichia coli* in some food products and human stool in Egypt. Veterinary World 10(10), 1233-1240.

Ibrahim, E.M.A., Sobeih, A.M.K., 2006. Diarrhoeagenic *E. coli* in kareish cheese manufactured by different methods with special reference to *Escherichia coli* O157: H7. Minufiya Veterinary Journal 4(1), 169-178.

Johnson, J.R., Russo, T.A., 2002. Extraintestinal pathogenic *Escherichia coli*: "the other bad *E. coli*". Journal of Laboratory and Clinical Medicine 139, 155–162.

Kaper, J.B., Nataro, J.P., Mobley, H.L., 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology 2, 123–140.

Kawano, K., Ono, H., Iwashita, O., Kurogi, M., Haga, T., Maeda, K., Goto, Y., 2012. Stx genotype and molec¬ular epidemiological analyses of Shiga toxin-producing *Escherichia coli* O157: H7/H in human and cattle isolates. European Journal of Clinical Microbiology and Infectious Diseases 31, 119-127.

Lahlaoui, H., Ben Haj Khalifa, A., Ben Moussa, M., 2014. Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum-lactamase (ESBL). Médecine et Maladies Infectieuses 44, 400–404.

Lamey, A.E., Ammar, A.M., Zaki, E.R., Khairy, N., Moshref, B.S., Refai, M.K., 2013. Virulence Factors of *Escherichia coli* Isolated from Recurrent Cases of Clinical and Subclinical Mastitis in Buffaloes. International Journal of Microbiological Research 4 (1), 86-94.

Mokady, D., Gophna, U., E. Z. Ron, E.Z., 2005. Extensive gene diversity in septicemic *Escherichia coli* strains. Journal Clinical Microbiology 43, 66–73.

Nataro, J.P. and Kaper, J.B. 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Review 11, 142–201.

Nazem, A.M., Ab Ishahid, Y.S., Abdel Aleem, S., 2016. Toxic-infectious bacteria in raw milk and soft cheese in local markets. Alexandria Journal of Veterinary Science 48(2), 91-95.

Ombarak, R.A., Hinenoya, A., Elbagory, A., Yamasaki, S., 2018. Prevalence and Molecular Characterization of Antimicrobial Resistance in *Escherichia coli* Isolated from Raw Milk and Raw Milk Cheese in Egypt. Journal of Food Protection 81(2), 226-232.

Ogutu, J., Zhang, Q., Huang, Y., Yan, H., Su, L., Gao, B., Zhang, W., Zhao, J., Cai, W., Li, W., Hong Zhao, H., Chen, Y., Song, W., Chen, X., Fu, Y., Zhang, F., 2015. Development of a multiplex PCR system and its application in detection of bla_{SHV}, bla_{TEM}, blaCTX-M-1, blaCTX-M-9 and $bla_{OXA\text{-}1}$ group genes in clinical Klebsiella pneumoniae and *Escherichia coli* strains. The Journal of Antibiotics 68, 725-733.

Perez, F.; Jones, H.; Hanson, N., Geyer, C. (2007): Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemotherapy 51, 3471–3484.

Rahal, E.A., Fadlallah, S.M., Nassar, F.J., Kazzi, N., Matar, G.M., 2015. Approaches to treatment of emerging Shiga toxin-producing *Escherichia coli* infections highlighting the O104:H4 serotype. Frontiers Cellular and Infection Microbiology 5, 24.

Rao, L.,Lv,L., Zeng, Z., Chen, S.,He,D., Chen, X., Wu,C., Wang, Y., Yang, T., Wu, P., Liu, Y., Liu, J.H., 2014. Increasing prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* in food animals and the diversity of CTX-M genotypes during 2003–2012. Veterinary Microbiology 172, 534–541.

Robins-Browne, R.M., Still, C.S., Miliotis, M.D., Richardson, N.J., Koornhof, H.J., Freiman. I., Schoub, B.D., Lecatsas, G., Hartman, E., 1980. Summer diarrhoea in African infants and children. Archives of Disease in Childhood 55, 923–928.

Ron, E. Z., 2006. Host specificity of septicemic *Escherichia coli*: human and avian pathogens. Current Opinion in Microbiology 9, 28–32.

Russo, T.A., Johnson, J.R. 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. Journal of Infection Diseases 181, 1753–1754.

Samadpour, M., Liston, J., Ongerth, J.E., Tarr, P.I., 1990. Evaluation of DNA probes for detection of shiga-like-toxin-producing *E. coli* in food and calf fecal samples. Applied Evironmental Microbiology 56, 1212-1215.

Sarimehmetoglu, B., Aksoy, M H., Ayaz, N.D., Ayaz, Y., Kuplulu, O., Kaplan, Y.Z., 2009. Detection of *Escherichia coli* O157:H7 in ground beef using immunomagnetic separation and multiplex PCR. Food Control 20, 357-361.

Slanec, T., Fruth, A., Creuzburg, K., Schmidt, H., 2009. Molecular analysis of virulence profiles and Shiga toxin genes in food-borne Shiga toxin-producing *Escherichia coli*. Applied Environmental Microbiology 75, 6187-6197.

Su, Y., Yu, C.Y., Tsai, Y., Wang, S.H., Lee, C.,Chu, C., 2014. Fluoroquinolone-resistant and extended-spectrum-lactamase-producing *Escherichia coli* from the milk of cows with clinical mastitis in Southern Taiwan. J Microbiology Immunology Infection 49, 892-901.

Tekiner, I.H., Özpınar, H., 2016. Occurrence and characteristics of extended spectrum beta-lactamases producing Enterobacteriaceae from foods of animal origin. Brazilian Journal of Microbiology 47, 444–451.

Wetzel, A.N. 2005. Studies in Shiga toxin-producing *Escherichia coli* O157:H7: determination of factors contributing to the dissemination of *Escherichia coli* O157:H7 among dairy farms. PhD Thesis, The Ohio State University.

Wilshaw, G.A., Cheasty, T., Smith, H.R., 2000. *Escherichia coli*. In: Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), The Microbiological Safety and Quality of Food II. Aspen Publishers, Inc., Gaithersburg, Maryland, pp. 1136-1177.