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Genetic Assessment of Shiga Toxin and Antibiotic Resistance of *E. coli* Isolated from Milk of Cows infected with Sub-clinical Mastitis

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Abstract

Bovine subclinical mastitis was one of the most important health problems facing dairy industry, its impact exceeded the economic aspects and extended to potential negative effects on human health. The current study aimed to determine the prevalence of *E. coli* as an important mastitic pathogen and identify some of its most important virulence gene as well as their antimicrobial resistance profile. In the present study *E. coli* was isolated and biochemically identified whereas out of 100 subclinically mastitic milk samples was nine samples were positive for *E. coli* with 9% prevalence rate. Serotyping of these isolates declared that 3 isolates were serotype O26:H11, 2 isolates in serotype O91:H21 and 1 isolate in each of serotypes O55:H7, O128:H2, O146:H21 and O124. Antimicrobial resistance profile of the obtained isolates showing that all the isolates were 100% resistant to both erythromycin and streptomycin, while 88.9% (8/9) were sensitive to gentamicin. The presence of 3 important virulence factors including Shiga toxin1(*Stx1*), Shiga toxin 2 (*Stx2*) and intimin (*eae*) genes, among the obtained isolates was reported using PCR. Molecular investigation revealing that 2 isolates contain all studied virulence genes (*Stx1*, *Stx2* and *eae*), 3 isolates contain (*Stx1* and *Stx2*), while *Stx1* was detected solely in 2 isolates, also 1 isolate contain only *Stx2* and lastly 1 isolate was negative for any of the studied virulence factors. In a conclusion, there was a 9% prevalence rate of *E. coli* in subclinically mastitic milk samples in the current study, indicating its importance as a mastitic pathogen. The Shiga toxin genes (*Stx1* & *Stx2*) are widely distributed among *E. coli* isolates, while the intimin (*eae*) gene is less prevalent in comparison to Shiga toxin genes. Also the recorded high multidrug resistance rate among the isolates posing threat to human health though entrance of these strains into the human being food chain whereas the isolated *E. coli* strains had the highest resistance to erythromycin and Streptomycin (100%), followed by Clindamycin (77.8%), Nalidixic acid (66.7%), and Gentamicin (11.1%) was the lowest.

KEYWORDS
Cow, Subclinical mastitis, *E. coli*, Shiga toxin, Antimicrobials.

INTRODUCTION

Subclinical mastitis is an important worldwide health problem in dairy farms accounts for several negative outcomes and significant changes in milk compositions. Economically, subclinical mastitis is an overemphasized form of mastitis due to its deleterious effect on both milk quality and production (Ruegg and Reinemann, 2002). Additionally, subclinical mastitis contributed to culling and increased risk of antibiotic residues in milk (McFadden, 2011). *Escherichia coli* (*E. coli*) is one of the most prevalent bacteria that had been linked to subclinical mastitis and antibiotic resistance (Hinthong *et al.*, 2017). *E. coli* is a Gram-negative opportunistic environmental bacterium that is most linked with mastitis in cattle (Fahim *et al.*, 2019). *E. coli* mastitis usually correlates with the bad hygienic measures in the animal environment (Abdel-Tawab *et al.*, 2018).

The most common serotypes of *E. coli* that recovered from mastitic milk were O55, O111, O124, O119, O114, O26, O157 and O44 (Momtaz *et al.*, 2012). Studies revealed that Shiga toxins (*Stx1* & *Stx2*) and intimin (*eae*) were the most significant virulence genes in *E. coli* strains isolated from bovine mastitic milk (Kobori *et al.*, 2004).

Shiga toxin-producing *E. coli* (STEC) were characterized by secretion of one or more Shiga toxins (*Stx1* or *Stx2*) that interfere with the synthesis of protein of host cell leading to the death of cells, these toxins called verotoxins due to their activities on vero cells or Shiga toxins due to their similarities with toxin of *Shigella dysenteriae* (Cookson *et al.*, 2006). STEC expresses another virulence factor called intimin, which facilitate bacterial adhesion and invasion into intestinal epithelial cells causing severe attaching-effacing lesion (Dhaka *et al.*, 2016). Intimin was encoded on *eae* chromosomal gene which is a part of pathogenicity island (Wang *et al.*, 2016).

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to most of the antimicrobial agents had become a growing problem (Levy, 1998), in addition to the detrimental impact on animal welfare and farm economics. Mastitis is greatly accounted for the use of different types of antimicrobials which in turn might result in establishment of resistant strains (Ismail and Abutarbush, 2020).

The elevation in antimicrobial resistance among *E. coli* either pathogenic or commensal, had been reported worldwide as a result of either overuse or misuse of antibiotics in animals and human (Szmolka and Nagy, 2013). Therefore, the current study aimed to determine the prevalence of *E. coli* as an important mastitic pathogen and to identify some of its most important virulence gene as well as their antimicrobial resistance profile.

MATERIALS AND METHODS

Milk samples

Milk samples were aseptically collected from lactating cattle in small holder farms of different localities in Assiut governorate, Egypt. The teat was washed, dried and the orifice was swabbed with 70% ethyl alcohol for disinfection. After the first few streams had been discarded, the milk samples were collected in sterile falcon tubes. All the milk samples were subjected firstly to California Mastitis Test (CMT) according to Schalm *et al.* (1971) and the CMT positive milk samples (n=100) were microbiologically tested in order to isolate the target pathogen.

Ethical Committee at Assiut University, Assiut, 868 Egypt, has approved all the procedures in this study in accordance with the Egyptian bylaws 869 and OIE animal welfare standards for animal care and use in research and education.

Isolation and identification of E. coli

Loopfuls from CMT positive milk samples (n=100) were enriched into MacConkey broth and incubated aerobically at 37°C for 24 h then loopful from the broth was cultured on MacConkey (MAC) agar (Merck, Germany) and incubated at 37°C, and bacterial growth was evaluated after 24 and 48 h. Gram negative micro-organisms were isolated from MAC agar and detected at the species level as putatively *E. coli* using oxidase, triple sugar iron agar, and IMViC tests (Franck *et al*., 1998).

Serological identification of E. coli

The isolates were serologically recognized according to Kok *et al.* (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for serotyping of the pathogenic types.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by single diffusion method according to Mary and Usha (2013) for the isolated *E. coli* strains. Sensitivity discs with variable concentrations were utilized to detect the susceptibility of the isolated pathogenic strains (Oxoid Limited, Basingstoke, Hampshire, UK). Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by Singh *et al.* (2010).

Polymerase Chain Reaction

Multiplex PCR according to Paton and Paton (1998) using specific primers (Table 1) was used in this study to investigate the presence of genes encoding Shiga toxins (*Stx1* and *Stx2*) and intimin (*eae*).

According to Sambrook *et al*. (1989), bacterial DNA was extracted using GeneJET Genomic DNA Purification Kit (Fermentas).

The amplification was conducted on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The reaction included 35 PCR cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35.

Amplified DNA fragments were electrophoresed on 2% agarose gel which was stained with ethidium bromide solution (0.5 µg/ml), visualized under an ultraviolet transilluminator and photographed.

Visible bands of appropriate size of 180 bp (*Stx1*), 255 bp (*Stx2*) and 384 bp (*eae*A) were considered positive. A 100 bp plus DNA Ladder (Fermentas) was used to determine the fragment sizes.

RESULTS

Out of 100 sub-clinical mastitic milk samples, 9 (9 %) were positive for presence of *E. coli*. Serodiagnosis of these 9 *E. coli* isolates revealing that they were belonging to serogroups O91: H21, O128: H2, O26: H11, O26: H11, O146: H21, O124, O91: H21, O26: H11 and O55: H7. Pathotypes characteristic of *E. coli* isolates were as the following: 5 isolates were enterohemorrhagic *E. coli* (EHEC), 2 isolates were enteropathogenic *E. coli* (EPEC), 1 strain was enteroinvasive *E. coli* (EIEC) and another 1 strain was enterotoxigenic *E. coli* (ETEC) (Table 2). The antimicrobial sensitivity testing indicated that the isolated *E. coli* strains had the highest resistance to erythromycin and Streptomycin (100%), followed by Clindamycin (77.8%), Nalidixic acid (66.7%), and Gentamicin (11.1%) was the lowest (Table 3).

The antimicrobial resistance pattern of different E coli serogroups isolated from sub-clinically mastitic milk showed that one isolates out of the three belonging to serogroups O26:H11 had the highest prevalence of antibiotic resistance (MAR index = 1), also one strain out of two below serogroups O91:H21 had a MAR index = 0.938, and lastly the lowest antimicrobial resistance were exhibited by isolates belong to serogroups O146:H21 and O124

Table 1. Primers used in the PCR

Primer	Oligonucleotide sequence (5'-3')	Product size	Reference
Stx1 (F)	**5′** ATAAATCGCCATTCGTTGACTAC **′3**	180	Paton and Paton (1998)
Stx1 (R)	**5′** AGAACGCCCACTGAGATCATC **′3**		
Stx2 (F)	**5′** GGCACTGTCTGAAACTGCTCC **′3**	255	
Stx2 (R)	**5′** TCGCCAGTTATCTGACATTCTG **′3**		
eaeA (F)	**5′** GACCCGGCACAAGCATAAGC **′3**	384	
eaeA (R)	**5′** CCACCTGCAGCAACAAGAGG **′3**		

as their MAR index was 0.125 (Table 4).

The results of PCR showing that 8 out of 9 (88.9%) isolates had at least one or more of the studied virulence genes (*Stx1*, *Stx2* and *eae*) (Table 5 and Fig. 1).

Table 2. Serological identification of *E. coli*

Identified Bacterium	Serodiagnosis	Strain characterization
E. coli	O91: H21	EHEC
	O128: H2	ETEC
	O26: H11	EHEC
	O26: H11	EHEC
	O146: H21	EPEC
	O124	EIEC
	O91: H21	EHEC
	O26: H11	EHEC
	O55: H7	EPEC

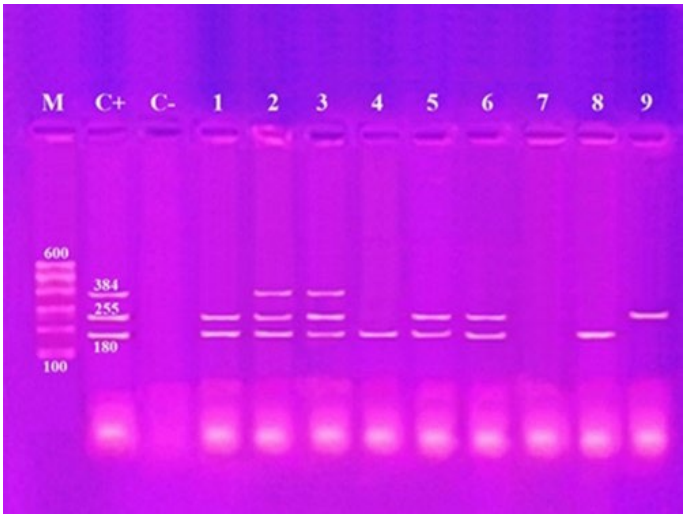

Fig. 1. Agarose gel electrophoresis of multiplex PCR of *Stx1* (180 bp), *Stx2* (255 bp) and *eae*A (384 bp) virulence genes for characterization of *E. coli*. Lane M: 100 bp ladder as molecular size DNA marker; Lane C+: Control positive *E. coli* for *Stx1*, *Stx2* and *eae*A genes; Lane C-: Control negative; Lanes 2 & 3 (O26): Positive strains for *Stx1*, *Stx2* and *eae*A genes; Lanes 1 (O26), 5 & 6 (O91): Positive strains for *Stx1* and *Stx2* genes; Lanes 4 (O55) & 8 (O128): Positive strains for *Stx1* gene; Lane 9 (O146): Positive strain for *Stx2* gene; Lane 7 (O124): Negative strain for *Stx1*, *Stx2* and *eae*A genes.

Table 3. Antimicrobial susceptibility of *E. coli* strains (n=9)

Antimicrobial agent	Antimicrobial susceptibility of *E. coli* strains					
	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Erythromycin (E)	-	-	-	-	9	100
Streptomycin (S)	-	-	-	-	9	100
Clindamycin (CL)	-	-	2	22.2	7	77.8
Nalidixic acid (NA)	2	22.2	2	22.2	6	66.7
Cefotaxim (CF)	3	33.3	1	11.1	5	55.6
Norocillin (NO)	4	44.4	-	-	5	55.6
Sulphamethoxazol (SXT)	2	22.2	3	33.3	4	44.4
Penicillin G (P)	4	44.4	1	11.1	4	44.4
Cephalothin (CN)	5	55.6	-	-	4	44.4
Oxytetracycline (T)	3	33.3	3	33.3	3	33.3
Doxycycline (DO)	5	55.6	1	11.1	3	33.3
Neomycin (N)	5	55.6	2	22.2	2	22.2
Ampicillin (AM)	5	55.6	2	22.2	2	22.2
Ciprofloxacin (CP)	6	66.7	1	11.1	2	22.2
Amikacin (AK)	6	66.7	1	11.1	2	22.2
Gentamicin (G)	8	88.9	-	-	1	11.1

Table 4. Antimicrobial resistance profile of *E. coli* strains (n=9)

NO	*E. coli* strains	Antimicrobial resistance profile	MAR index
1	O26: H11	E, S, CL, NA, CF, NO, SXT, P, CN, T, DO, N, AM, CP, AK, G	1
2	O26: H11	E, S, CL, NA, CF, NO, SXT, P, CN, T	0.625
3	O26: H11	E, S, CL, NA	0.25
4	O91: H21	E, S, CL, NA, CF, NO, SXT, P, CN, T, DO, N, AM, CP, AK	0.938
5	O91: H21	E, S, CL	0.187
6	O55: H7	E, S, CL, NA, CF, NO, SXT, P, CN	0.562
7	O128: H2	E, S, CL, NA, CF, NO	0.375
8	O146: H21	E, S	0.125
9	O124	E, S	0.125
		Average	0.472

E: Erythromycin; S: Streptomycin. CL: Clindamycin; NA: Nalidixic acid; CF: Cefotaxim; NO: Norocillin; SXT: Sulphamethoxazol; P: Penicillin-G; CN: Cephalothin; T: Oxytetracycline; DO: Doxycycline; N: Neomycin; AM: Ampicillin; CP: Ciprofloxacin; AK: Amikacin; G: Gentamicin.

Table 5. Occurrence of virulence genes in *E. coli* isolated from the examined samples.

E. coli serovars	No. of ex. isolates	virulence genes in *E. coli*					
		Stx_1		Stx_2		*eaeA*	
		NO.	%	NO.	%	NO.	%
O26: H11	3	3	100	3	100	2	66.7
O55: H7	1	1	100	0	0	0	0
O91: H21	2	2	100	2	100	0	0
O124	1	0	0	0	0	0	0
O128: H2	1	1	100	0	0	0	0
O146: H21	1	0	0	1	100	0	0

Stx1: Shiga- toxin 1 gene; *Stx2*: Shiga- toxin 2 gene; *eae*A: Intimin gene.

DISCUSSION

E. coli is one of important mastitic pathogens so the studying of its virulence factors and antimicrobial susceptibility is greatly required. In the present study, *E. coli* was isolated and biochemically identified in 9 out of 100 milk samples, with 9% prevalence rate, from lactating cows with subclinical mastitis. A similar prevalence rate (9.3%) was reported by Ombarak *et al*. (2019). Also, Lira *et al*. (2004); Momtaz (2010); Abdel-Tawab *et al*. (2018) isolated *E. coli* from 8.5%, 10.5% and 7.5% of the tested milk samples respectively. On other side, Momtaz *et al*. (2012) found that 57 of 181 mastitic milk samples tested were positive for *E. coli*, with percentage about 31.5%. Variations in the frequency of *E. coli* isolation were noticed between different countries and regions which may be attributed to differences in climate, lactation season, diet and various managemental conditions (Marashifard *et al*., 2019).

It was found that the isolates belonged to serotypes O26:H11 (3/9 isolates), O91:H21 (2/9), O55:H7 (1/9), O128:H2 (1/9), O146:H21 (1/9) and O124 (1/9). Momtaz *et al*. (2012) reported that 19.2%, 15.8%, 12.3%, 12.3%, 10.5%, 7%, 7%, and 3.5% of the *E. coli* isolates were belonging to the O55, O111, O124, O119, O114, O26, O157, and O44 serogroups. Also Osman *et al*. (2012) showed that, from 40 mastitic milk samples, 77.4% of the isolates belonged to four different O serogroups (O26, O86, O111, and O127). In case of *E. coli* mastitis, serotyping was not of greatest importance as there isn't predominant serotype specifically isolated (Abdel-Tawab *et al*., 2018).

Results of antimicrobial sensitivity testing showed that resistance to Erythromycin (E), Streptomycin (S), Clindamycin (CL) and Nalidixic acid (NA) was the highest. Multidrug resistance was stated among 152 isolates (65.8%) (Stephan *et al*., 2008). Langoni *et al*. (2000) reported a discrete level of resistance to tetracycline (13.0%) and ampicillin (12.0%) among *E. coli* isolates from bovine mastitis. The high rate of antibiotic resistance showed by the isolated *E. coli* strains in this study was a logical result of the haphazard and uncontrolled use of antibiotics in veterinary practice in Egypt. This might contribute to the emergence of resistant strains that might be transmitted to humans by entering their food chain.

In the present study, the results of multiplex PCR showed that 8 out of 9 *E. coli* isolates (88.9%) were STEC; however, 2 of these STEC (25%) were positive also for the presence of *eae* gene. Infection by STEC clinically elicited by varying degree of diarrhea which might be watery or bloody and hemolytic uremic syndrome (HUS). Shiga toxin (stx) was a potent cytotoxin responsible for severe tissue damage (Gyles, 2007).

Virulence gene encoding *Stx1* was more prevalent in the current *E. coli* isolates (77.8%, 7/9), followed by gene encoding *Stx2* (66.7%, 6/9) and lastly *eae* gene (22.2%, 2/9). Currently, the *eae* gene was detected only in isolates harboring *Stx1* and *Stx2* genes. Salvadori *et al*. (2003) detected the presence of *eae* gene in percent about 21.2% among Shiga toxin producing *E. coli* isolates. The *eae* gene was detected in both STEC and non STEC (Kobori *et al*., 2004). About 93% of *E. coli* isolates carried Shiga toxin 1 virulence gene and only 68% of them carried intimin gene (Ismail and Abutarbush, 2020). Osman *et al*. (2012) revealed that all *E. coli* strains that had been isolated from mastitic milk samples had *Stx1*, *Stx2*, *hyl*A, *Flic*(h7), *stb, F41, K99, sta, F17, LT-I, LT-II*, and *eae*A virulence genes. Moreover, Moussa *et al*. (2010) confirmed that the *Stx2* and *eae*A genes were the most prevalent virulence factors in cow's environment that was contaminated by feces, and it was also a frequent cause of bovine mastitis.

Intimin (*eae*) gene accounted for the ability of *E. coli* to attach and effacement (A/E) the intestinal epithelial cells (Aidar-Ugrinovich *et al*., 2007). Intimin (*eae*) gene was detected in 14 out of 42 (33.3%) *E. coli* isolates (Momtaz, 2010). Variations in the types and distribution of virulence genes demonstrated in *E. coli* isolates might be attributed to differences in study areas. Geographical situation might govern the presence of different virulence genes in *E. coli* isolates (Momtaz, 2010).

CONCLUSION

There is a 9% prevalence rate of *E. coli* in subclinically mastitic milk samples in the current study. The Shiga toxin genes (*Stx1* & *Stx2*) are widely distributed among *E. coli* isolates, while the intimin (*eae*) gene is less prevalent in comparison to Shiga toxin genes. Also, the recorded high multidrug resistance rate among the isolates posing threat to human health though entrance of these strains into the human being food chain whereas the isolated *E. coli* strains have the highest resistance to erythromycin and Streptomycin (100%), followed by Clindamycin (77.8%), Nalidixic acid (66.7%), and Gentamicin (11.1%) is the lowest.

CONFLICT OF INTEREST

Authors declares that they have no competing interests to disclose.

REFERENCES

Abdel-Tawab, A.A., El-Hofy, F.I., El-Ekhnawey, K.I., El-Shenawey, F.A., 2018. Studies on Echericia coli isolated from mastitic cattle and comparative revealance to human. Int. J. Adv. Res. 6, 1206-1215.

Aidar-Ugrinovich, L., Blanco, J., Blanco, M., Blanco, J.E., Leomil, L., Dahbi, G., Morab, A., Onuma, L., Silveiraa, W.D., Pestana de Castro, A.F., 2007. Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in São Paulo, Brazil. Int. J. Food Microbiol. 115, 297-306.

Cookson, A.L., Taylor, S.C.S., Bennett, J., Thomson-Carter, F., Attwood, G.T., 2006. Serotypes and analysis of distribution of Shiga toxin-producing *Escherichia coli* from cattle and sheep in the lower North Island, New Zealand. New Zealand. NZ. Vet. J. 54, 78–84.

Dhaka, P., Vijay, D., Vergis, J., Negi, M., Kumar, M., Mohan, V., Doijad, S., Poharkar, K.V., Malik, S.S., Barbuddhe, S. B., Rawool, D.B., 2016. Genetic diversity and antibiogram profile of diarrhoeagenic *Escherichia coli* pathotypes isolated from human, animal, foods and associated environmental sources. Infect. Ecol. Epidemiol. 6, 31055.

Fahim, K.M., Ismael, E., Khalefa, H.S., Farag, H.S., Hamza, D.A., 2019. Isola-

tion and characterization of *E. coli* strains causing intramammary infections from dairy animals and wild birds. Int. J. Vet. Sci. Med. 7, 61-70.

Franck, S.M., Bosworth, B.T., Moon, H.W., 1998. Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves. J. Clin. Microbiol. 36, 1795-1797.

Gyles, C.L. 2007. Shiga toxin-producing *Escherichia coli*: an overview. J. Anim. Sci. 85, E45-E62.

Hinthong, W., Pumipuntu, N., Santajit, S., Kulpeanprasit, S., Buranasinsup, S., Sookrung, N., Chaicumpa, W., Aiumurai, P., Indrawattana, N., 2017. Detection and drug resistance profile of *Escherichia coli* from subclinical mastitis cows and water supply in dairy farms in Saraburi Province, Thailand. Peer J. 5, e3431.

Ismail, Z.B., Abutarbush, S.M., 2020. Molecular characterization of antimicrobial resistance and virulence genes of *Escherichia coli* isolates from bovine mastitis. Vet. World 13, 1588-1593.

Kobori, D. Rigobelo, E.C., Macedo, C., Marin, J.M., Avila, F.A., 2004. Virulence Properties of Shiga Toxin-Producing *Escherichia coli* Isolated from Cases of Bovine Mastitis in Brazil. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux 57, 15-20.

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

Langoni, H., de Araejo, W.N., da Silva, A.V., de Souza, L.C., 2000. Tratamento da mastite bovina com amoxicilina e enrofloxacina bem como com a sua associated. Arquivos do Instituto Biologico 67, 177-180.

Levy, S.B., 1998. The challenge of antibiotic resistance. Sci. Am. 278, 46-53.

Lira, W.M., Macedo, C., Marin, J.M., 2004. The incidence of Shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. J. Appl. Microbiol. 97, 861-866.

Marashifard, M., Aliabad, Z.K., Hosseini, S.A.A.M., Daraban-Sarokhalil, D., Mirzaii, M., Khoramrooz, S.S., 2019. Determination of antibiotic resistance pattern and virulence genes in *Escherichia coli* isolated from bovine with subclinical mastitis in southwest of Iran. Trop. Anim. Health Prod. 51, 575-580.

Mary, C., Usha, M., 2013. Incidences of multi-drug resistance *Escherichia coli* isolates in Panipuri sold in Bangalore. Int. Food Res. J. 20, 1007-1009.

McFadden, M., 2011. California mastitis test and milk quality. Michigan Dairy Review 16, 2.

Momtaz, H., 2010. Investigation of virulence factors in *Escherichia coli* isolated from clinical and subclinical bovine mastitis. B.J.V.M. 13, 122–126.

Momtaz, H., Safarpoor Dehkordi, F., Taktaz, T., Rezvani, A., Yarali, S., 2012. Shiga toxin-producing *Escherichia coli* isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. Scientific World Journal 2012, 618709.

Moussa, I.M., Ashgan, M.H., Alwathnani, H.A., Mohamed, K.F., Al-Doss, A.A., 2010. Multiplex polymerase chain reaction for detection and characterization of Shiga toxigenic *Escherichia coli* (STEC). Afr. J. Biotechnol. 9, 4356–4363.

Ombarak, R.A., Zayda, M.G., Awasthi, S.P., Hinenoya, A., Yamasaki, S., 2019. Serotypes, Pathogenic Potential, and Antimicrobial Resistance of *Escherichia coli* Isolated from Subclinical Bovine Mastitis Milk Samples in Egypt. Jpn. J. Infect. Dis. 72, 337–339.

Osman, K.M., Mustafa, A.M., Aly, M.A.K., AbdElhamed, G.S., 2012. Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* isolated from mastitic milk relevant to human health in Egypt. Vector-Borne Zoonot. Dis. 12, 297–305.

Paton, J.C., Paton, A.W., 1998. Pathogenesis and diagnosis of Shiga- toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. 11, 450-479.

Ruegg, P.L., Reinemann, D.J., 2002. Milk quality and mastitis tests. Bovine Practice 36, 41-54.

Salvadori, M.R., Valadares, G.F., Leite, D.S., Blanco, J., Yano, T., 2003. Virulence factors of *Escherichia coli* isolated from calves with diarrhea in Brazil. Braz. J. Microbiol. 34, 230-235.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: Laboratory Manual. 2nd ed. Cold spring Harbor. New York. USA.

Schalm, O.W., Carrol, E.J., Jain, N.C., 1971. Bovine Mastitis. Lea and Febriger. Philadelphia. USA.

Singh, A., Yadav, S., Singh, S., Bharti, P., 2010. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Int. 43, 2027-2030.

Stephan, R., Schumacher, S., Corti, S., Krause, G., Danuser, J., Beutin, L., 2008. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in Swiss raw milk cheeses collected at producer level. J. Dairy Sci. 91, 2561-2565.

Szmolka, A., Nagy, B., 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Front. Microbiol. 4, 258. https://doi.org/10.3389/fmicb.2013.00258

Wang, J., Stanford, K., McAllister, T.A., Johnson, R.P., Chen, J., Hou, H., Zhang, G., Niu, Y.D., 2016. Biofilm Formation, Virulence Gene Profiles, and Antimicrobial Resistance of Nine Serogroups of Non-O157 Shiga Toxin-Producing *Escherichia coli*. Foodborne Pathog. Dis. 13, 316-324.