Journal of Animal Research: v.12 n.04, p. 575-581. August 2022

DOI: 10.30954/2277-940X.04.2022.17

Characterization of *E. coli* Isolates from Meat Samples for Shiga Toxin Producing Virulence Markers

Chinta Siva Swetha[1], Porteen Kannan[1*], A. Elango[2], B.S.M. Ronald[3], T.M.A. Senthil Kumar[4] and Amal Rose, T.F.[1]

[1]*Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai, Tamil Nadu, INDIA*
[2]*Dean, Veterinary College and Research Institute, Thalaivasal Koot Road, Salem, Tamil Nadu, INDIA*
[3]*Department of Veterinary Microbiology, Madras Veterinary College, Chennai, Tamil Nadu, INDIA*
[4]*Zoonoses Research Laboratory, Centre for Animal Health Studies, TANUVAS, Chennai, INDIA*

[*]*Corresponding author: K Porteen; E-mail: rajavet2002@gmail.com*

Received: 20 May, 2022 **Revised:** 08 July, 2022 **Accepted:** 12 July, 2022

ABSTRACT

The presence study was designed to identify shiga toxin producing *E. coli* Non O157:H7 isolates from meat samples, to detect the presence of virulence markers and to characterize isolates for ESBL resistance by PCR. A total of 150 meat samples (50 of each beef, chicken, and mutton) were collected from retail outlets in Chennai and subjected to conventional and molecular methods for isolation of *E. coli.* Out of 150 samples, 71 presumptive *E. coli* isolates recovered by conventional method, among which 61 isolates were confirmed by PCR targeting *uspA*. All these 61 isolates were tested for presence of virulence markers *viz., stx1, stx2, eae*, *hlyA* by multiplex PCR and found 45 isolates were harboured the virulence markers either in combination or alone. Further, all the shiga toxin *E. coli* isolates were streaked on CT-SMAC and found that 21 isolates had shown pink colour colonies representing them as *E. coli* non O157:H7. PCR assay of ESBL resistance genes revealed that 40 (66 %), 7 (11.5%), 37(60.7%) *E. coli* isolates were harbouring CTX-M gene, SHV and TEM genes. Presence of virulence markers in *E. coli* Non O157:H7 isolates coupled with ESBL resistance is a real threat to food safety and consumer point of view which warrants the need for devising intervention strategies to combat virulent and anti-microbial resistant *E. coli* at both household and industrial level.

HIGHLIGHTS

- Prevalence of *E. coli* non O157:H7 isolates in meat samples.
- Detection of virulence markers and ESBL antibiotic resistance among *E. coli* non O157:H7 isolates.
- Distribution of ESBL antibiotic resistance genes indicates the pathogenic potential of *E. coli* non O157:H7 isolates.

Keywords: *E. coli,* Virulence markers, Shiga toxin, Meat, ESBL resistance

Now-a-days, animals are raising solely for meat production as the meat and meat products are becoming an important and nutritious component in human diet and they are also associating with transmission of hazards which causes disease and death (Chaudhary *et al.,* 2014). WHO has defined as foodborne diseases are those associated with the ingestion of contaminated food which causes a wide spectrum of illness (Chaudhary *et al.,* 2014). WHO has estimated that 30% of population in developed countries were suffering from food borne diseases each year whereas 2 million deaths are estimated per year in developing countries (Chaudhary *et al.,* 2014). *Escherichia coli*, a common inhabitant in humans and animal intestines are gram negative, rod shaped, flagellated, non-sporulating, facultative anaerobe belongs to the family Enterobacteriaceae (Momtaz *et al.,* 2013). This intestinal

How to cite this article: Swetha, C.S., Kannan, P., Elango, A., Ronald, B.S.M., Senthil Kumar, T.M.A. and Amal Rose, T.F. (2022). Characterization of E. coli Isolates from Meat Samples for Shiga Toxin Producing Virulence Markers. *J. Anim. Res.,* **12**(04): 575-581.

Source of Support: None; **Conflict of Interest:** None

organism can double within 20 minutes and it can easily disseminate in to different ecosystems through the various routes like water, soil, food and others (Chaudhary *et al.,* 2014).

Most of the *E. coli* isolates are harmless but some isolates are of public health importance that causes gastrointestinal illness in man (Momtaz *et al.,* 2013). Among them, Shiga toxin producing *E. coli* is considered as toxin producing and the most significant food borne pathogen (Panahee and Pourtaghi, 2016). Though the *E. coli* O157:H7 is the most common serogroup related to food or food borne diseases, but the CDC has estimated that non O157:H7 serotypes are responsible for one third of STEC infections in US.

Ruminants especially cattle and sheep are the primary reservoirs of STEC but there are many reports that it has been isolated from deer, horse, dogs and birds. Meat contaminated with STEC during slaughter is the principal route by which the pathogens can enter the meat supply chain (Barlow *et al.,* 2006). However, contact with infected animals, consumption of contaminated water and contact with animal manure are indirect routes of transmission of STEC.

The pathogenicity of STEC is due to a number of virulence factors including shiga toxins (*stx1* and *stx2*), intimin protein (*eae*) which is responsible for attachment of STEC to intestinal epithelial cells and enterohemolysin (*hly*A) (Momtaz *et al.,* 2013). These shiga toxins can cause wide variety of illnesses from uncomplicated diarrhoea to hemorrhagic colitis and can progress in to haemolytic uremic syndrome (HUS) which includes micro-angiopathic haemolytic anaemia, thrombocytopenia and severe acute renal failure (Momtaz *et al.,* 2013; ECDC, 2011).

The intensive and indiscriminate use of antibiotics in veterinary and medical fields resulted in development of antibiotic resistance in many number of enteric pathogens which is considering as major global health issue both from the health and wealth aspect (Ibrahim *et al.,* 2016). Extended spectrum beta-lactamase (ESBL) producing *E. coli* is classified by WHO as Superbugs which has the potential to inactivate differentbeta-lactam antibiotics. In the past, ESBL producing *E. coli* isolates were reported from nosocomial infections, but in recent times, ESBL producing *E. coli* outbreaks were also reported from community infections with Zoonotic potential (Oteo *et al.,* 2010). Detection of ESBL producing *E. coli* in food producing animals and edible animal products has become a serious cause of concern in food safety (Geser *et al.,* 2012). These problems posed a challenge to both medical and veterinary practitioners to face the serious consequences on the treatment of these infectious diseases.

Keeping this in view the present study was designed to isolate and identify Shiga toxin producing *Escherichia coli* Non O157:H7 isolates from meat samples and to elucidate the pathogenic potential by identification of virulence markers using specific primers and to identify the antibiotic resistance pattern in the *E. coli* isolates for ESBL by genotypic methods.

MATERIALS AND METHODS

Sampling site and Size

A total of 150 meat samples (Fifty of each chicken, mutton and beef samples) were collected from retail outlets in Chennai. Approximately, 10 g of each of different fresh raw meat samples were collected in sterile polythene zip lock packs under aseptic conditions and transferred immediately on to ice packs and transported to the Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai for further analysis.

Isolation and identification of *E. coli* by cultural method

The samples were enriched in nutrient broth and incubated for 18-24 hrs at 37ºC. The enriched samples were streaked on Eosin Methylene Blue (EMB) agar and the plates were incubated at 37ºC for 18-24 hrs. The presumptive colonies were stored in glycerol stock for future analysis.

Detection of *E. coli* and identification of virulence markers by Polymerase Chain Reaction

Boiling and snap chilling method was employed for DNA extraction. The PCR amplification was optimized by using 25 µl PCR reaction mixture which consists of 3 µl of Template DNA, each 1 µl of 10 pmol primers (forward and reverse), 12.5 µl of 2X PCR master mix and 7.5 µl Nuclease Free Water (NFW) in Eppendorf thermal cycler under standardized using standardized cycling

conditions and suitable primers (Table 1 and Table 2). The PCR products were further analysed by Agar Gel Electrophoresis.

Differentiation of *E. coli* non-O157:H7 and *E. coli* O157:H7 on CT-SMAC agar

All the shiga toxin harbouring *E. coli* isolates were streaked on CT-SMAC agar and incubated at 37ºC for 18-24 hrs.

Genotypic characterization for ESBL

Genotypic characterization for ESBL was carried out by PCR targeting 3 different genes (CTX-M, TEM, SHV) by using specific primers given in Table 3 and the standardized cycling conditions were mentioned in Table 4. The PCR products were analysed by agarose gel electrophoresis.

RESULTS AND DISCUSSION

Isolation and identification of *E. coli*

A total of 71 presumptive isolates with greenish metallic sheen on EMB agar plate were obtained from 150 meat samples. On further confirmation with PCR targeting *usp*A gene, 61 samples (40.6%) were confirmed as *E. coli,* among which 16 (32%) isolates were obtained from

Table 1: Primers used for molecular identification of *E. coli* and its virulence genes

Targetgene	Sequences (5' to 3')	Size (bp)	References
usp A	F: CCG ATA CGC TGC CAA TCA GT	884	Chen and Griffiths, 1999
	R: ACG CAG ACC GTA GGC CAG AT		
Stx1	F: ATA AAT CGC CAT TCG TTG ACT AC	180	
	R: AGA ACG CCC ACT GAG ATC ATC		
Stx2	F: AGA ACG CCC ACT GAG ATC ATC	255	
	R: TCG CCA GTT ATC TGA CAT TCT G		
eae	F: GAC CCG GCA CAA GCA TAA GC	385	Bandyopadhya *et al.* 2012
	R: CCA CCT GCA GCA ACA AGA GG		
hlyA	F: GCA TCA TCA AGC GTA CGT TCC	534	
	R: AAT GAG CCA AGC TGG TTA AGC T		

Table 2: Cycling conditions for identification of *E. coli* and its virulence genes

Steps	*usp A*	mPCR for *Stx1,Stx2, eae, hlyA*	No. of cycles for *uspA*	No. of cycles for virulence genes
Initial Denaturation	94ºC for 5 min	95ºC for 5 min	1	1
Denaturation	94ºC for 1 min	94ºC for 45sec		
Primer annealing	55ºC for 1 min	59ºC for 45 sec	30	30
Extension	72ºC for 2 min	72ºC for 1 min		
Final extension	72ºC for 5 min	72ºC for 6 min	1	1

Table 3: Primers used in this study for ESBL resistance

Resistance gene	Sequences (5' to 3')	Size (bp)	Reference
CTX-M	F: CGC TTT GCG ATG TGC AG	590	Amiri *et al.* 2016
	R: ACC GCG AA TCG TTG GT		
SHV	F: GAT GAA CGC TTT CCC ATG ATG	214	
	R: CGC TGT TAT CGC TCA TGG TAA		Shacheraghi *et al.* 2009
TEM	F: ATG AGT ATT CAA CAT TTC CG	847	
	R: GTC ACA GTT ACC AAT GCT TA		

Table 4: Cycling conditions for the ESBL resistance genes

ESBL resistance genes	Cycling conditions				
	Initial Denaturation	**Denaturation**	**Annealing**	**Extension**	**Final Extension**
CTX-M	94ºC for 5 min	94ºC for 25 s Repeat for 30 cycles	52ºC for 40 s	72ºC for 50 s	72ºC for 6 min
SHV	94ºC for 5 min	94ºC for 60 s Repeat for 35 cycles	61ºC for 60 s	72ºC for 60 s	72ºC for 5 min
TEM	96ºC for 5 min	96ºC for 60 s Repeat for 35 cycles	58ºC for 60 s	72ºC for 60 s	72ºC for 10 min

Table 5: Distribution of Virulence markers in *E. coli* isolates from different meat samples

Virulence markers	Beef	Mutton	Chicken	Total
only *Stx1*	0	0	0	0
only *Stx2*	1	3	3	7
only *eae*	3	7	0	10
only *hlyA*	1	0	2	3
stx1 + stx2	1	0	0	1
Stx1 + eae	0	0	0	0
Stx1 + hlyA	1	0	0	1
Stx1 + eae + hly A	0	0	0	0
stx2 + eae	0	4	1	5
stx2 + hly A	9	1	4	14
stx2 + eae + hly A	0	1	0	1
stx1 + stx2 + eae	0	0	0	0
stx1 + stx2 + hlyA	*1*	0	0	1
eae + hlyA	*2*	0	0	2
stx1 + stx2 + eae + hly A	0	0	0	0
Total	**19**	**16**	**10**	**45**

chicken, 21 from mutton (42%), 24 from beef (48%). These findings are similar to the findings of Khan *et al.* (2002) (49.5%) in India, Panahee and Pourtaghi (2016) (39.5%) in Iran. On contrary, Sharma *et al.* (2016) and Shekh *et al.* (2013) from India had reported a very high (98%) and very low (8.8%) prevalence rates than the present study. The variations in the prevalence rates of *E. coli* from meat samples reported by different researchers might due to poor personal hygiene, quality of water used for processing and improper or no sanitation of the knives as well as working surfaces in the retail outlets.

On virulence gene profiling, it was found that all 61 *E. coli* isolates obtained in the present study had harboured *stx1*, *stx2*, *eae* and *hlyA* virulence genes either in single or in a combination as follows: 7, 10, 3 isolates were harbouring only *stx*2, *eae* and *hly*A whereas only 1 isolate for each *stx*1 + *stx*2 and *stx*1 + *hly*A, 5 and 14 for *stx*2 + *eae*, *stx*2 + *hly* A, 2 for *eae* + *hly*A, 1 for each *stx*2 + *eae* + *hly*A and *stx*1 + *stx*2 + *hly*A; none of the isolates showed *stx*1 in single (Table 5). Among them, thirty-two *E. coli* isolates were identified as STEC (52.4%) as they harboured *stx*1 or *stx*2 virulence marker, of which eight isolates were from chicken, nine from mutton and 15 isolates from beef. Whereas Selim *et al.* (2013) reported 2 isolates harbouring only *stx*1 from meat samples and none of the isolates have *stx*2, *eae* and *hly*A or combination of them. Victoria *et al.* (2013) also reported the frequency of different virulence markers such as 62% of *stx*2, 35% of *stx*1+*stx*2 and 3 % of *stx*1. Panahee and Pourtaghi (2016) also reported 72.4% *stx*2, 13.7% *eae*, 6.9% *stx*2 and *eae,* 6.9% of isolates harbouring *stx*1, *stx*2 and *eae*.

Our result showed that *stx*2 is more prevalent than *stx*1 and was high from beef samples which are similar to the findings of Victoria *et al.* (2013) and Panahee and Pourtaghi (2016). It was clear from previous investigations that *stx*2 is 1000 times more dangerous than *stx*1; a combination

Table 6: ESBL resistance patterns of *E. coli* isolates isolated in different meat samples

Type of sample	Total No. of E. coli samples by PCR	TEM		SHV		CTX-M	
		n	%	n	%	n	%
Chicken	16	11	68.8	0	0	10	63
Mutton	21	11	52.4	2	9.5	18	86
Beef	24	15	62.5	5	20.8	12	50
Total	**61**	**37**	**60.7**	**7**	**11.5**	**40**	**66**

of *stx*2 with *eae* is more potent and is a predictor of HUS (Ethelberg *et al.,* 2004). In contrary to the present study findings, Hameed *et al.* (2017) and Selim *et al.* (2013) reported that *stx*1 is more prevalent than *stx*2 whereas Dombrosio *et al.* (2007) reported that none of his STEC isolates harboured *stx1* or *stx*2.

Our study reported that eight (16%) STEC isolates were identified from chicken which was almost similar to the investigations of Minh *et al.* (2015). This may be opined as poultry meat may also be one of the reservoirs for STEC pathogen in addition to ruminant meat (Dipineto *et al.,* 2006). In beef samples, fifteen (30%) STEC isolates were recognized which was similar to the findings of Momtaz *et al.* (2013) (29.7%), Panahee and Pourtaghi, (2016) (23.5%) and was higher than the findings of Brooks *et al.* (2001) in New Zealand (12%) and Fantelli and Stephan, (2001) in Switzerland (1.75%). Nine (18%) mutton samples in this study have contaminated with STEC isolates which is similar to the findings of Brooks *et al.* (2001) in New Zealand (17%) and was higher than the studies of Franco *et al.* (2009) (7.1%) in Italy and lower than the findings of Momtaz *et al.* (2013) (35.4%) in Iran. Our results correlates with the findings of other workers and it also documents the presence of shiga toxin genes from various meats irrespective various geographical location.

All the 32 STEC isolates were further streaked on CT-SMAC agar to differentiate the *E. coli* Non O157:H7 from *E. coli* O157:H7. Twenty-one isolates (65.6 %) showed pink colour colonies which indicates Shiga toxin producing *E. coli* Non O157:H7 whereas eleven isolates (36.4%) shown colourless colonies on CT-SMAC agar. In the past more concern on *E. coli* O157:H7 serotype was studied extensively for its pathogenic potential, however, in the present day scenario, researchers have encountered more number of STEC Non O157:H7 isolates from raw meats which is also documented in our study.

Out of 32 STEC isolates, 28% of beef samples (nine isolates), 12.5% of chicken (4 isolates) and 25 % of mutton samples were contaminated with *E. coli* non-O157:H7. In contrast to this, Patrica *et al.* (2014) reported very high incidence of *E. coli* Non O157 (80.7%) and *E. coli* O157 (19.2%) from 57 STEC isolates in beef whereas Li *et al.* (2011) recorded a very low prevalence of 5.7%, 4% *E. coli* non O157:H7 from beef, chicken samples, similar prevalence from beef (4%) and with zero prevalence of *E. coli* O157:H7. It is similar to the findings of Minh *et al.* (2015) who also reported 4.4% of *E. coli* non-O157:H7 and zero prevalence of *E. coli* O157:H7 from meat samples. Results of our study and study throughout the world have recorded the presence of shiga toxins from *E. coli* non O157:H7 isolates which highlighted the pathogenic potential that needs strict vigilance at all the levels of production chains to prevent food borne outbreaks.

Genotypic characterization of *E. coli* isolates for ESBL was carried out by targeting 3 different genes namely TEM, SHV and CTX-M and the prevalence of resistance pattern for various ESBL gene targets are listed in Table 6. ESBL resistance patterns among the isolates reported the prevalence rate of 66%,60.7% and 11.5% for CTX-M, TEM, SHV genes respectively, which is similar to the findings of Radhika *et al.* (2017) who had also reported 77.36% of CTX-M, 54.7% of TEM and 62.26% SHV from milk, milk products and meat whereas Geser *et al.* (2012) in Zurich reported 85.7% of CTX-M and 2.2% of TEM from chicken faecal samples. Food borne pathogens with transmissible drug resistance genes may have a profound effect on the future treatment options for a wide range of infections involving these gram-negative bacteria.

CONCLUSION

The presence of *E. coli* in meat samples collected from retail outlets was high which indicates poor hygiene

practices prevailing in slaughter, transport and distribution. Distribution of various virulence markers and ESBL antibiotic resistance highlights the pathogenic potential of *E. coli* non-O157:H7 isolates which emphasises the need for routine screening at various stages of production chains at household and industrial levels to ensure food safety to consumers.

ACKNOWLEDGEMENTS

This research was funded by Tamil Nadu Veterinary and Animal Science University.

REFERENCES

Amiri, H.S., Reza,R., Sohrabi, N. and Khamesipou, F. 2016. Molecular Typing of Uropathogenic *Escherichia coli* Strains isolated from patients by REP-PCR. *Sovremennye Tehnologiiv Medi.,* **9**(3): 71.

Khan, A., Yamasaki, S., Sato, T., Ramamurthy, T., Pal, A., Datta, S., Chowdhury, N.R., Das, S.C., Sikdar, A., Tsukamoto, T. and Bhattacharya, S.K. 2002. Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef, and humans, Calcutta, India.*Emer. Inf. Dis*., **8**(1): 54-62.

Bandyopadhyay, S., Lodh, C., Rahaman, H., Bhattacharya, D., Bera, A.K., Ahmed, F.A., Mahanti, A., Samanta, I., Mondal, D.K., Sarkar, S. and Dutta, T.K. 2012. Characterization of shiga toxin producing (STEC) and enteropathogenic *Escherichia coli* (EPEC) in raw yak (*Poephagus grunniens*) milk and milk products. *Res. Vet. Sci.*, **93**(2): 604-610.

Barlow, R.S., Gobius, K.S. and Particia M. Desmarchelier. 2006. Shiga toxin-producing *Escherichia coli* in ground beef and lamb cuts: Results of a one-year study. *Int. J. Food Microb.,* **111**: 1-5.

Brooks, H.J., Mollison, B.D., Bettelheim, K.A., Matejka, K., Paterson, K.A. and Ward, V.K. 2001. Occurrence and virulence factors of non-O157 Shiga toxin-producing *Escherichia coli* in retail meat in Dunedin, New Zealand. *Lett. Appl. Microbiol*., **32**(2): 118-22.

Chaudhary, S., Khurana, S.K. and Mane, B.G. 2014. *Escherichia coli*: Animal Foods and Public Health-Review. *J. Microb., Imm. Biotech*., **1**: 31-46.

Chen, J. and Griffiths, M.W. 1999. Cloning and sequencing of the gene encoding universal stress protein from *Escherichia coli* O157:H7 isolated from Jack-in-a-Box outbreak. *Lett. Appl. Microb*., **29**: 103–107.

Dambrosio, A., Lorusso, V., Quaglia, N.C., Parisi, A., La Salandra, G., Virgilio, S., Mula, G., Lucifora, G., Celano, G.V. and Normanno, G. 2007. *Escherichia coli* O26 in minced beef: prevalence, characterization and antimicrobial resistance pattern. *Int. J. Food Microbiol.*, **118**(2): 218-22.

Dipineto, L., Santaniello, A., Fontanella, M., Lagos, K., Fioretti, A. and Menna, L.F. 2006. Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. *Lett. Appl. Microbiol.*, **43**(3): 293-5.

Ethelberg, S., Olsen, K.E., Scheutz, F., Jensen, C., Schiellerup, P., Engberg, J., Petersen, A.M., Olesen, B., Gerner-Smidt, P. and Mølbak, K. 2004. Virulence factors for hemolytic uremic syndrome, Denmark. *Emer. Inf. Dis*., **10**(5): 842.

European Centre for Disease Prevention Control (ECDC)/ European Food Safety Authority (EFSA) 2011. Shiga toxin/ verotoxin-producing *Escherichia coli* in humans, food and animals in the EU/EEA, with special reference to the German outbreak strain STEC O104. *In:* J. Takkinen, M. Struelens, T. Niskanen, P. Makela, V. Rizzi, A. Caprioli, & F. Scheutz (Eds.), Microbiology of Shiga toxin/verotoxin producing *Escherichia coli* (pp. 2) (Stockholm).

Fantelli, K. and Stephan, R. 2001. Prevalence and characteristics of Shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *Int. J. Food Microb*., **70**(1-2): 63-69.

Franco, A., Lovari, S., Cordaro, G., Di Matteo, P., Sorbara, L., Iurescia, M., Donati, V., Buccella, C. and Battisti, A. 2009. Prevalence and concentration of verotoxigenic *Escherichia coli* O157: H7 in adult sheep at slaughter from Italy. *Zoonoses Pub. Health*, **56**(5): 215-220.

Geser, N., Stephan, R. and Hachler, H. 2012. Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet. Res*., **8**: 21.

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

Ibrahim, D.R., Christine, E.R. Dodd, Dov J. Stekel, Stephen J. Ramsden and Jon L. Hobman. 2016. Multidrug resistant, extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from a dairy farm. *FEMS Microbiol. Ecol.,* **92**(4): fiw013.

Radhika,K.R., Porteen, K., Selvaraju, G. and Ramesh, S. 2017. Characterization of extended-spectrum β lactamase producing *Escherichia coli* and *Salmonella* in foods of animal origin. MVSc Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University.

Li, M.C., Fang Wang, and Fan Li. 2011. Identification and Molecular Characterization of Antimicrobial-Resistant Shiga

Toxin–Producing *Escherichia coli* Isolated from Retail Meat Products. *Foodborne Pathog. Dis.,* **8**(4): 489–493.

Minh, S.H., Etsuko Kimura, Duc Hoang Minh, Ken-ichi Honjoh and Takahisa Miyamoto. 2015. Virulence characteristics of Shiga toxin-producing *Escherichia coli* from raw meats and clinical samples. *Microb. Immun*. **59**(3): 114-22.

Momtaz, H. and Jamshidi, A. 2013. Shiga toxin –producing *Escherichia coli* isolated from chicken meat in Iran: Serogroups, virulence factors, and antimicrobial resistance properties. *Poult. Sci.*, **92**: 1305-1313.

Momtaz, H., Farhad, S., Dehkordi, E.R., Hossein E. and Reza A. 2013. Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminant's meat. *Meat Sci*., **95**: 381-388.

Oteo, J., Bautista, V., Lara, N., Cuevas, O., Arroyo, M., Fernandez, S., Lazaro, E., de Abajo, F.J. and Campos, J. 2010. Parallel increase in community use of fosfomycin and resistance to fosfomycin in extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. *J. Antimicrob. Chemother.*. **65**: 2459-2463.

Panahee, M. and Pourtaghi, H. 2016. Virulence gene profiles of shiga-toxin producing *Escherichia coli* isolates from retail raw meat in Iran. *Bulg. J. Vet. Med.* ISSN 1311-1477. DOI: 10.15547/bjvm.953.

Patricia Llorente, Laura Barnech, Kinue Irino, Maria Valeria Rumi and Adriana Bentancor. 2014. Characterization of Shiga Toxin producing *Escherichia coli* isolates from ground beef collected in different socio-economic strata markets in Buenos Aires, Argentina. *BioMed Res. Int*., **92**(4): fiw013.

Selim, S.A., Ahmed, S.F., Aziz, M.H.A., Zakaria, A.M., Klena, J.D. and Pangallo, D. 2013. Prevalence and characterization of Shiga-toxin O157: H7 and non-O157: H7 enterohemorrhagic *Escherichia coli* isolated from different sources. *Biotech. Biotech. Equip.*, **27**(3): 3834-3842.

Shahcheraghi, F., Siavash Nasiri and Hanieh Noveiri, 2009. Detection of extended-spectrum β-lactamases (ESBLs) in *Escherichia coli*. *Iran. J. Clin. Infectious Dis.,* **4**(2): 65-70.

Sharma, G., Sharma, S., Sharma, P., Chandola, D., Dang, S., Gupta, S. and Gabrani, R. 2016. *Escherichia coli* biofilm: development and therapeutic strategies. *J. Appl. Microbiol.*, **21**(2):309-19.

Shekh, C.S., Deshmukh, V.V., Waghamare, R.N., Markandeya, N.M. and Vaidya, M.S. 2013. Isolation of pathogenic *E. coli* from buffalo meat sold in Parbhani city, Maharashtra, India. *Vet. World*, **6**(5):277-279.

Victoria Brusa, Virginia Aliverti, Florencia Aliverti, Emanuel E. Ortega, Julian H. de la Torre, Luciano H. Linares, Marcelo E. Sanz, Analía I. Etcheverría, Nora L. Padola, Lucía Galli, Pilar Peral García, Julio Copes and Gerardo A. Leotta. 2013. Shiga toxin-producing *Escherichia coli* in beef retail markets from Argentina. *Front. Cell. Infec. Microb.,* **2**: 171.