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Molecular characterization of *Escherichia coli* isolated from meat and meat products

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Abstract
This study was aimed at the molecular characterization of *Escherichia coli* isolated from meat and meat products. A total of 100 samples comprising of chicken meat (30), mutton (20), chevon (20) and RTE meat products (30) were collected from Udaipur city (Rajasthan). The prevalence of *E. coli* were recorded in chicken meat, mutton, chevon and RTE meat products as 56.66% (17), 45% (9), 40% (8) and 13.33% (4), respectively. Out of total 38 *E. coli* isolates, 12 isolates were found to be multi drug resistant. On molecular profiling, all the 12 MDR isolates were found to be positive for *uidA* gene. Out of the 12 isolates, 16.66% (2) were positive for virulence gene (*stx1*). The prevalence of *bla*TEM and *bla*OXA antibiotic resistant genes was observed as 83.33% (10) and 8.33% (1), respectively.

Keywords: Molecular characterization, *Escherichia coli*, meat and RTE meat products

Introduction
Escherichia coli is a member of Enterobacteriaceae family. It is a short, Gram negative, non-spore forming and motile bacterium with peritrichous flagella. It is a facultative anaerobic bacterium. Poultry industries are most vulnerable to the attack by *E. coli* that has increased the mortality of poultry (Kaper and Sperandio, 2005) [21]. On the basis of their virulence, *E. coli* is classified into six categories namely enterohaemorrhagic *E. coli*, also called shiga toxin-producing *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli* and diffusely adherent *E. coli* (Darwish *et al.*, 2015) [9]. *E. coli* strains are usually recovered from intestinal tracts of human, poultry, animals and commonly present in soil, water and foods due to faecal contamination or contamination during food animal slaughter (Udaykar *et al.*, 2009) [37]. *E. coli* produces three distinct enterotoxins; heat labile toxins (LT), heat stable toxins (ST) and verotoxins (VT). Verotoxins are also called as Shiga-like toxin (SLT). *E. coli* can cause haemorrhagic colitis, severe food poisoning, hemolytic uremic syndrome, bloody diarrhea, non-bloody diarrhea, abdominal cramps, vomiting, dysentery, kidney infection, septicemia, pneumonia and meningitis (Gupta *et al.*, 2013) [14]. About 5-10% of the infected people may develop haemolytic uremic syndrome, a life-threatening kidney disease with kidney failure-like symptoms (Sherikar *et al.*, 2011; CDC, 2018) [31, 8].
The PCR is a rapid and reliable tool for the molecular based diagnosis of *E. coli* infections. The *uidA* gene encodes an enzyme beta-glucuronidase are that works on the analysis of sugars. The presence of this gene has been investigated in *E. coli* bacteria. The virulence genes are ideal targets for the determination of the pathogenic potential of any given *E. coli* isolate (Farhan and Jubeer 2018) [12]. Presence of virulence factors including shiga toxins (*stx1* and *stx2*) in the STEC strains of meat products make them lethal pathogens for public health. These genes are responsible for bacterial adhesion, colonization and invasion into the gastric epithelial cells (Shahreza *et al.*, 2017) [30].
In India, ESBL-positive strains of *E. coli* have been isolated from clinical samples of humans but studies on *E. coli* of livestock and poultry origin are limited (Bhave *et al.*, 2019) [5]. In addition, several studies investigated the transfer of ESBL producers from poultry to humans and suggested chicken as a reservoir of ESBL genes, plasmid or clones as a risk for humans (Casella *et al.*, 2017) [7].

The ESBL genes are located on plasmids that can be easily transferred between and within bacterial species (Bush and Jacoby, 2010) [6]. The emergence of ESBL producing *E. coli* in the food-producing animals and in foods of animal origin is a growing problem worldwide There has been an increase in beta-lactam resistance in *E. coli* in the last few years due to production of ESBL.

Materials and Methods

Samples

A total of 100 samples comprising of chicken meat (30), mutton (20), chevon (20) and RTE meat products (30) were collected from Udaipur city (Rajasthan). The samples of meat and ready to eat meat products were collected twice in a week from meat outlets and shops from Udaipur city in Rajasthan. The samples were collected in sterile container and transported to the laboratory within 2 hours in chilled condition by using ice packs.

Molecular characterization

Isolation of DNA from pure culture was undertaken using by Nucleo-pore gDNA fungal/bacterial mini kit by following the manufacturer's instructions supplied along with the kit. Genomic DNA isolated from *E coli* isolates were used in the PCR. Published primers were used for the detection of *uidA*, *stx1*, *bla*TEM and *bla*OXA gene in *E. coli* isolates are described in Table No. 1.

Table 1: The primers used for detection of *uidA*, *stx1*, *bla*TEM and *bla*OXA gene

S. No.	Oligo Name	Sequence (51-31)	Size of amplified product (bp)	Reference
1.	*uidA*	F-GCGTCTGTTGACTGGCAGGTGGTGG	510	Johnson *et al.*, 2017 [19]
		R-GTTGCCCGCTTCGAAACCAATGCCT		
2.	*Stx1*	F-CAGTTAATGTGGTGGCGAAG	894	Hazarika *et al.*, 2007 [17]
		R-CTGCTAATAGTTCTGCGCATC		
3.	*blaTEM*	F-GAGTATTCAACATTTTCGT	857	Maynard *et al.*, 2003 [25]
		R-ACCAATGCTTAATCAGTGA		
4.	*blaOXA*	F-GCAGCGCCAGTGCATCAAC	198	Maynard *et al.*, 2003 [25]
		R-CCGCATCAAATGCCATAAGTG		

F= Forward, R= Reverse

The PCR procedure to screen the *uidA*, *stx1*, *blaTEM* and *blaOXA* gene in *E. coli* isolates was standardized as described by Johnson *et al.*, 2017, Hazarika *et al.*, 2007, Maynard *et al.*, 2003 [19, 17, 21, 25] with certain modifications. Followed by preliminary trials, the reaction mixture was optimized to contain 12.5 µl 2X PCR master mix, 10 nmol of each forward and reverse primer, 10.5 µl nuclease free water and 1 µl of DNA template. The reaction was performed in the thermal cycler with pre-heated lid (lid temp. =105°C). The cycling conditions of *uidA*, *stx1*, *blaTEM* and *blaOXA* gene were comprised of 30 cycles of denaturation, annealing and extension which are described in Table No 2.

Table 2: Steps and conditions of thermal cycling for different primer pairs in PCR

Primers		Cycling conditions			
(Forward and Reverse)	**Initial denaturation**	**Denaturation**	**Annealing**	**Extension**	**Final extension**
uidA(F) *uidA*(R)	94 ºC 5 minutes	94 ºC 1 minute	67 ºC 1 minute	72 ºC 1.5 minutes	72ºC 5 minutes
Repeated for 30 cycles					
stx1 (F) *stx1*(R)	94ºC 5 minutes	94 ºC 1 minute	55 ºC 1 minute	72 ºC 1.5 minutes	
Repeated for 30 cycles					
bla_{OXA} (F) bla_{OXA} (R)	94 ºC 5 minutes	94 ºC 1 minute	50 ºC 1 minute	72 ºC 1.5 minutes	72ºC 5 minutes
Repeated for 30 cycles					
bla_{TEM} (F) bla_{TEM}(R)	94 ºC 5 minutes	94 ºC 1 minute	51 ºC 1 minute	72 ºC 1.5 minutes	
Repeated for 30 cycles					

Results and Discussion

Out of the 38 isolates, 12 MDR E. coli isolates were selected for molecular characterization by targeting the virulence and resistance genes. First of all, detection of *uidA* gene was done by standardizing the PCR protocol as per the method described by Johnson *et al.*, 2017 [19]. Electrophoresis analysis revealed a specific amplification of 510 bp product of the *uidA* gene. In all the multidrug resistant isolates collected from the different sources of meat samples like chicken meat, mutton, chevon and RTE meat products, 12 isolates were found to be positive for *uidA* gene. The *uidA* gene encodes an enzyme β-D -glucuronidase that works on the analysis of sugars. The most commonly use flourogenic substrate for the detection of E. coli is 4-methylumbelliferyl- β-D-glucuronide (Manafi *et al.*, 1991 and Feng *et al.*, 1982) [24, 13]. β-D-glucuronidase activity in *E. coli* is induced by a variety of β-D -glucuronidase, among which methyl glucuronidase is the most effective (Stoeber 1961) [33]. Bej *et al.*, 1991 [4] have shown that *uidA* gene sequence is unique to *E. coli*. However, the primers specific to this region also amplifies few species of *Shigella*. Heijnen and Medema 2006 [18] have shown that *uidA* gene was detected in *E. coli* as well as *Shigella* species. In our study, all the test isolates confirmed as *E. coli* by biochemical tests were also found positive for *uidA* gene which was in accordance to the study conducted by Albarri *et al.*, 2017 [2].

Further, the detection of virulence gene (*stx1*) was carried out as per the method described by Hazarika *et al.*, 2007 [17]. Shiga toxin (*Stx*) is one of the most potent bacterial toxins that are produced in some strains of *E. coli*. It is similar to AB type toxin that has a binding unit and a catalytically active unit. It is associated with impairment of protein synthesis in

target cells by inhibition of ribosomal function (Melton-Celsa 2014) [26]. Shiga-toxin producing *E. coli* infections are an important public health hazard. STEC infection mainly occurs through the consumption of contaminated food and water (Karmali 2004) [23]. The shiga toxin producing *E. coli* is an important virulence factor which leads to the most serious manifestation of the disease, the hemolytic uremic syndrome (HUS) which is more often associated with strains that produce *stx1*. Shiga toxin leads to the pathogenesis by inducing microvascular changes *in vivo* and are cytotoxic to selected cell lines *in vitro*. Intimin helps in the pathogenesis by adherence to intestinal villi and entero-hemolysis may enhance the effect of shiga toxins (Kang *et al.,* 2004) [20]. Shiga toxigenic *E. coli* strains are important especially O157: H7 which is recognized as emerging food borne pathogen (Gyles 2007) [15]. The animals especially sheep and goat have been implicated as the reservoir of STEC (Doregiraee *et al*., 2016) [10]. Out of the 12 MDR isolates, only two (16.66%) isolates were found positive for *stx1* gene (one each from chicken and mutton. Similar prevalence rates of *stx1* gene were reported previously by Silva *et al*., 2011, Dutta *et al*., 2011 and Tahamtan *et al*., 2010 [32,11,35] as 13.3%, 19.04% and 12.14%, respectively.

PCR assay for the detection of *bla*TEM and *bla*OXA genes in *E. coli* was standardized with primers reported by Maynard *et al.,* 2003 [25], with slight modifications. PCR assay revealed a specific amplification of 857 bp and 198 bp product for *bla*TEM and *bla*OXA genes. The exposure of *E. coli* strains to β-lactams has led to dynamic and massive production and mutation of β-lactamases. High usage of antibiotics in animal farms is responsible for the high prevalence of ESBL producing *E. coli*. The prevalence of ESBL *E. coli* in different types of meat even in organic meat has been reported in several countries (Stuart *et al.,* 2012) [34]. *E. coli* is an indicator organism for antimicrobial resistance in the members of Enterobacteriaceae. The emergence of extended spectrum β-lactamase in livestock and poultry is a global concern. In India, the studies on *E. coli* of livestock and poultry origin are limited (Kar *et al.,* 2015) [38]. The *bla*TEM enzymes spread worldwide and are found in Enterobacteriaceae family. ESBL is divided into four main groups from A to D. ESBL enzymes *TEM*, *SHV* and *CTX-M* from group A are reported to be produced by *E. coli*. These enzymes can hydrolyze ampicillin, carbenicillin, oxacillin and other cephalosporins (Paterson and Bonomo 2005) [28]. In the present study, out of the 12 MDR isolates, 10 (83.33%) were found positive for *bla*TEM which included nine isolates from chicken meat and one from chevon. Similar findings were previously reported by Ali *et al.*,2018, Hakim *et al.,* 2017 and Abd *et al.,* 2015 [3,16,1] who reported the prevalence as 66.7%, 71.4% and 73.33%, respectively. On the other hand, the *bla*OXA group was considered as the first group of *OXA*-type beta-lactamases conferring carbapenemase resistance and has been reported worldwide. The *bla*OXA in *E. coli* is very rare and unique (Paul *et al.,* 2017) [29]. In the present study, among the 12 MDR isolates, only one isolate from chicken meat (8.33%) was found to be positive for *bla*OXA. Similar findings were previously reported by Bhave *et al.,* 2019, Moawad *et al.,* 2017 and Talukdar *et al.,* 2013 [5, 27, 36] who revealed the prevalence as 8.69%, 14.3% and 8.33%, respectively.

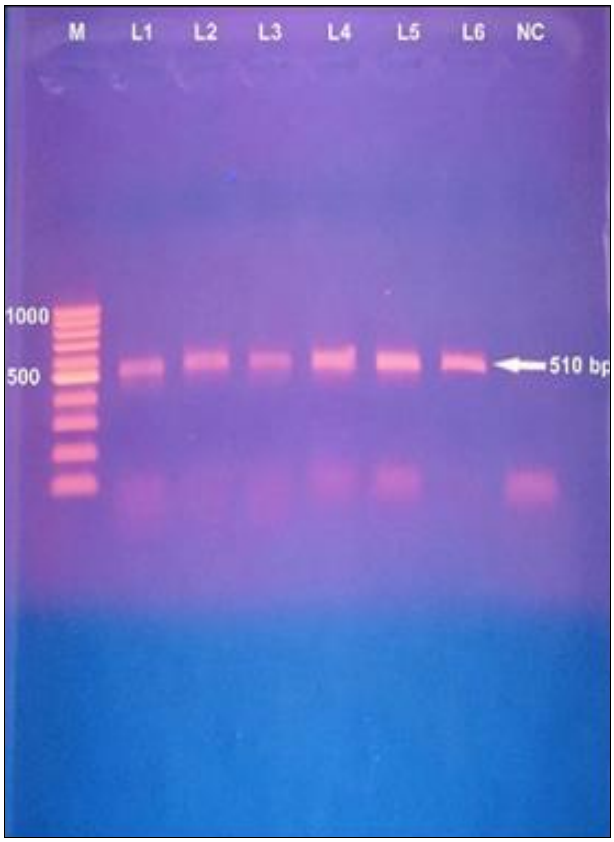

Fig 1: Agarose gel showing PCR amplified product (510 bp) for *uidA* gene in the test isolates. M=1000kb DNA ladder, positive samples (L1=C-22, L2= C-15, L3= C-5, L4= C-20, L5= C-16, L6= C-13, NC= negative control)

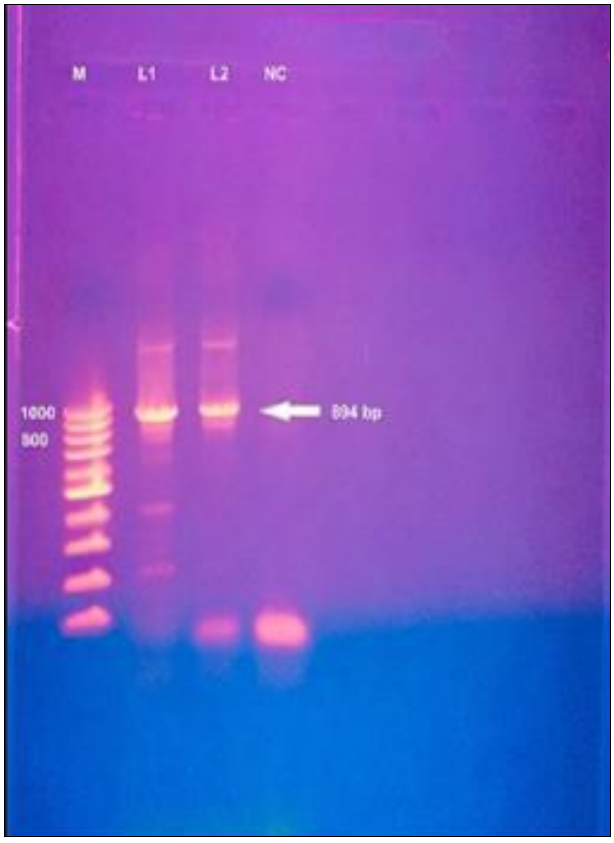

Fig 2: Agarose gel showing PCR amplified product (894 bp) for *stx1* gene in MDR *E. coli* isolates. M =1000kb DNA ladder, positive samples (L1=C-29, L2=S-20, NC= negative control)

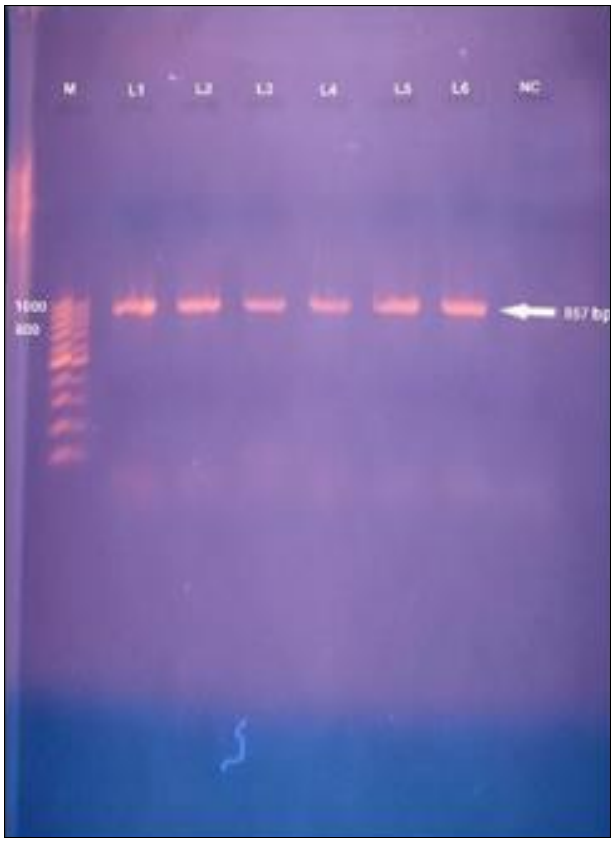

Fig 3: Agarose gel showing PCR amplified product (857 bp) for *bla*TEM gene in MDR *E. coli* isolates. M =1000kb DNA ladder, positive samples (L1=C-22, L2= C15, L3= C-5, L4= C-20, L5=C-16, L6= C-13, NC= negative control)

Conclusion

In the current study, out of the 38 isolates, 12 MDR *E. coli* isolates were selected for molecular characterization by targeting the virulence and resistance genes. Firstly, detection of *uidA* gene was done by standardizing the PCR protocol. In all the multidrug resistant isolates collected from the different sources of meat samples like chicken meat, mutton, chevon and RTE meat products, all the MDR isolates (12) were found to be positive for *uidA* gene. Further, the detection of virulence gene (*stx1*) was carried out. Out of the 12 MDR isolates, only two (16.66%) isolates were found positive for *stx1* gene (one each from chicken and mutton). Similarly, out of the 12 MDR isolates, 10 (83.33%) were found positive for *bla*TEM which included nine isolates from chicken meat and one from chevon. While, among the 12 MDR isolates only one isolate from chicken meat (8.33%) was found to be positive for *bla*OXA. Thus, the high prevalence of multidrug resistant *E coli* isolates with virulence genes is a matter of concern for the public health. So, the antibiotics should be used judiciously in animal husbandry practice to prevent the emergence of antibiotic resistant bacterial strains.

References

1. Abd ET, Ammar AAM, Nasef SA, Reda RM. Antibacterial resistance and resistance gene detriments of E. coli isolated from chicken. Benha Veterinary Medical Journal. 2015;28(2):231-240.
2. Albarri OM, Var I, Meral M, Heshmati B, Koksal F. Prevalence of *Escherichia coli* isolated from meat, chicken and vegetable samples in Turkey. Journal of Biotechnology Science Research. 2017;4(3):214-222.
3. Ali R, Shehedi A, Eraqi M. Molecular studies on *Escherichia coli* isolated from diarrheic dog. Int. J Pharm. Sci. Rev. Res. 2018;50(1):1-8.
4. Bej AK, Dicesare JL, Haff L, Atlas RM. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for uid A. Appl. Environ. Microbiol. 1991;57(4):1013-1017.
5. Bhave S, Kolhe R, Mahadevaswamy R, Bhong C, Jadhav S, Nalband S, *et al*. Phylogrouping and antimicrobial resistance analysis of extraintestinal pathogenic *Escherichia coli* isolated from poultry species. Turkish Journal of Veterinary and Animal Sciences. 2019;43(1):117-126.
6. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrobial Agents and Chemotherapy. 2010;54(3):969-976.
7. Casella T, Nogueira MCL, Saras E, Haenni M, Madec JY. High prevalence of ESBL in retail chicken meat despite reduced use of antimicrobials in chicken production, France. International Journal of Food Microbiology. 2017;257(1):271-275.
8. Centers for Disease Control and Prevention (CDC). Outbreak of E. coli infections linked to ground beef, 2018. [Online] Available at: https://www.cdc.gov/ecoli o26-09-18/index.html. Accessed 3 March 2018.
9. Darwish WS, Eldin WFS, Eldesoky KI. Prevalence, molecular characterization and antibiotic susceptibility of *Escherichia coli* isolated from duck meat and giblets. Journal of Food Safety. 2015;35(3):410-415.
10. Doregiraee F, Alebouyeh M, Fasaei BN, Charkhkar S, Tajedin E, Zali MR. Isolation of atypical enteropathogenic and shiga toxin encoding *Escherichia coli* strains from poultry in Tehran, Iran. Gastroenterology and Hepatology from Bed to Bench. 2016;9(1):53-57.
11. Dutta TK, Roychoudhury P, Bandyopadhyay S, Wani SA, Hussain I. Detection & characterization of shiga toxin producing *Escherichia coli* (STEC) and enteropathogenic E. coli (EPEC) in poultry birds with diarrhoea. The Indian Journal of Medical Research. 2011;133(5):541-545.
12. Farhan MB, Jubeer OJ. Molecular study of some virulence factors enteropathogenic *Escherichia coli* isolated from new-born till the age of one year. Journal of Pharmaceutical Sciences and Research. 2018;10(12):3388-3392.
13. Feng PC, Hartman PA. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. 1982;43(6):1320-1329.
14. Gupta B, Ghatak S, Gill JPS. Incidence and virulence properties of E. coli isolated from fresh fish and ready-to-eat fish products. Veterinary World. 2013;6(1):5-9.
15. Gyles CL. Shiga toxin-producing *Escherichia coli*: An overview. Journal of Animal Science. 2007;85(13):45-62.
16. Hakim AS, Omara ST, Syame SM, Fouad EA. Serotyping, antibiotic susceptibility, and virulence genes screening of *Escherichia coli* isolates obtained from diarrheic buffalo calves in Egyptian farms. Veterinary World. 2017;10(7):769-773.
17. Hazarika RA, Singh DK, Kapoor KN, Agrawal RK, Pandey AB, Purosottam. Verotoxigenic *Escherichia coli* (STEC) from beef and its products. Ind. J of Experimen. Biology. 2007;45(2):207-211.
18. Heijnen L, Medema G. Quantitative detection of E. coli O157 and other shiga toxin producing E. coli in water

samples using a culture method combined with real-time PCR. Journal of Water and Health. 2006;4(4):487-498.
19. Johnson JR, Johnston BD, Gordon DM. Rapid and specific detection of the *Escherichia coli* sequence type 648 complex within phylogroups. F. Journal of Clinical Microbiology. 2017;55(4):1116-1121.
20. Kang SJ, Ryu SJ, Chae JS, Eo SK, Woo GJ, Lee JH. Occurrence and characteristics of enterohemorrhagic *Escherichia coli* O157 in calves associated with diarrhoea. Veterinary Microbiology. 2004;98(3-4):323-328.
21. Kaper JB, Sperandio V. Bacterial cell-to-cell signalling in the gastrointestinal tract. Infection and Immunity. 2005;73(6):3197-3209.
22. Saleh KJ, Alakiu DM, Adam AS. Search for *E. coli* o157:h7 in stool samples from diahrreagenic patients attending dutsin-ma general hospital, Katsina state, north-western Nigeria. Int. J Biol. Sci. 2021;3(1):34-40. DOI: 10.33545/26649926.2021.v3.i1a.27
23. Karmali MA. Infection by shiga toxin-producing *Escherichia coli*. Molecular Biotechnology. 2004;26(2):117-122.
24. Manafi Mohammed, Kneifel Wolfgang, Bascomb Shoshana. Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiology and Molecular Biology Reviews. 1991;55(3):335-348.
25. Maynard C, Fairbrother JM, Bekal S, Sanschagrin FO, Levesque RC, Brousseau R, *et al*. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* o149: k91 isolates obtained over a 23-year period from pigs. Antimicrobial Agents and Chemotherapy. 2003;47(10):3214-3221.
26. Melton-Celsa AR. Shiga toxin (stx) classification, structure and function. Microbiology Spectrum. 2014;2(2):1-21.
27. Moawad AA, Hotzel H, Awad O, Tomaso H, Neubauer H, Hafez HM *et al*. Occurrence of *Salmonella enterica* and *Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. Gut Pathogens. 2017;9(1):57.
28. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clinical Microbiology Reviews. 2005;18(4):657-686.
29. Paul D, Ingti B, Bhattacharjee D, Maurya AP, Dhar D, Chakravarty A, Bhattacharjee A. An unusual occurrence of plasmid-mediated blaOXA-23 carbapenemase in clinical isolates of *Escherichia coli* from India. International Journal of Antimicrobial Agents. 2017;49(5):642-645.
30. Shahreza MHS, Rahimi E, Momtaz H. Shiga-toxigenic *Escherichia coli* in ready to eat food staffs: Prevalence and distribution of putative virulence factors. Microbiology Research. 2017;8(2):88-92.
31. Sherikar AT, Bachhil VN, Thapliyal DC. Textbook of element of veterinary public health, published by Dr. T. P Trivedi, project director (DKMA), Directorate of knowledge management in agriculture, ICAR, Krishi Anusandhan Bhavan, Pusa, New Delhi; c2011.
32. Silva IMM, Evencio-Neto J, Silva RM, Lucena-Silva N, Magalhaes J, Baliza M. Genotypically characterization of *Escherichia coli* isolates from poultry. Arq. Bras. Med. Vet. Zootec. 2011;63(2):333-339.
33. Stoeber F. These de Docteur des Sciences University of Paris, Paris, France. 1961;17: 89-140.
34. Stuart JC, van den Munckhof T, Voets G, Scharringa J, Fluit A, Leverstein-Van Hall M. Comparison of ESBL contamination in organic and conventional retail chicken meat. International journal of Food Microbiology. 2012;154(3):212-214.
35. Tahamtan Y, Hayati M, Namavari MM. Prevalence and distribution of the stx1, stx2 genes in shiga toxin producing E. coli (STEC) isolates from cattle. Iranian Journal of Microbiology. 2010;2(1):8-13.
36. Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM *et al*. Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. PloS One. 2013;8(4):1-8.
37. Udaykar A, Sharda R, Chhabra D, Sharma V. Study of virulence factors of *Escherichia coli* strains isolated from diarrhoeic calves. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 2009;30(1):26-28.
38. Kar D, Bandyopadhyay S, Bhattacharyya D, Samanta I, Mahanti A, Nanda PK *et al*. Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India. Infection, Genetics and Evolution. 2015;(29):82-90.