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Enumeration, Serotypes and Virulence Genes Associated with Shigatoxigenic (STEC) and Enterotoxigenic (ETEC) *Escherichia coli* from Beef and Chicken of Mizoram, India

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ABSTRACT

Background: *E. coli* is one of the most important zoonotic pathogen and also an indicator of faecal contamination of food and water. There is paucity of data regarding the level of contamination of raw meat and characterization of pathogenic *E. coli* from slaughtered cattle and chicken in un-organized sector from Mizoram, India.

Methods: Raw meat samples from traditionally slaughtered cattle and chicken were collected from Aizawl, Kolasib and Champhai districts of Mizoram and analyzed for *E. coli* count (ECC), serotypes and virulence genes of STEC and ETEC.

Result: A proportion of 65.55 per cent beef and 58.89 per cent chicken had unacceptable level of ECC. The most predominant serotypes of *E. coli* were O118 (13.33%) in beef and O8 (13.89%) in chicken. Both STEC (8.00% and 6.94%) and ETEC (12.00% and 26.38%) pathotypes were detected in beef and chicken, respectively. Detection of serotype O121 in chicken and O26 and O111 in both beef and chicken along with virulence genes of STEC indicated the contamination of raw meat with highly pathogenic STEC strains.

Key words: *Escherichia coli*, ETEC, Retail meat, STEC, Virulence genes.

INTRODUCTION

Food-borne infections and zoonoses are major causes of illness and death worldwide with subsequent economic losses and thus constitute one of the threat multiplier for food safety and security costing billions of dollars in morbidity and mortality. Diarrhoeal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230 000 deaths every year (Brooks *et al.,* 2021).

Amongst the approximately 200 different types of food borne diseases, bacterial contamination is the most common cause; some are even capable of producing heat-resistant toxins, accounting for more than 70 per cent of deaths associated with food borne transmission (Thomas, 2017). India is prone to food borne problems because of climatic conditions, food habits, poverty, inadequate basic hygienic and sanitary facilities and low public awareness of food safety and lack of a risk-based food control system to reduce food borne diseases in conventional food production settings (WHO, 2020). Food can be contaminated at any point of production and distribution and the primary responsibility lies with food producers and handlers.

A large proportion of the world's population relies on meat as a source of protein (Bradeeba and Sivakumaar, 2013). North-East region of India is inhabited by different ethnic tribes, where majority of the populations consume meat as an important component of their diet. There is ever increasing demand of meat in Mizoram. Beef and chicken are predominantly consumed meat besides pork which is produced in unorganized sector and usually sold in the retail markets. The meat available at retail outlets comes through a long chain of slaughtering and transportation where each step may pose a risk of microbial contamination.

Escherichia coli is a member of coliform group of bacteria and its presence in food is indicative of faecal contamination of food and water, poor hygienic conditions or existence of enteric pathogens and *E. coli* itself is the most important zoonotic food borne pathogen (Gozde and Emec, 2019). Some of the *E. coli* strains are highly pathogenic in human and animals and people with low immunity (Akbar *et al*., 2014).

The unhygienic production, processing and selling environment of meat bear high chances of bacterial

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contamination of meat. Rapid and simultaneous detection of food-borne pathogens is a key step towards ensuring food safety and can be easily facilitated by PCR (Radji *et al.,* 2010). The present paper describes the enumeration of *E. coli*, its serotypes and STEC and ETEC pathotypes in raw meat from conventionally slaughtered cattle and chicken of Mizoram, India.

MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences and Animal Husbandry, Aizawl, Mizoram during 2019-20.

A total of 180 raw meat samples, 90 each of beef and chicken, were collected randomly from conventionally slaughtered cattle and chicken on weekly Saturday market days from three districts, Aizawl, Kolasib and Champhai, of Mizoram state of India. The *E. coli* count (ECC) was determined on Hicrome *E. coli* agar using spread plate technique and the number of colonies in appropriate dilution was recorded as the total number of bacteria (cfu/ g) = Average number *E. coli* colony count on the plates × 10 × dilution factor. The *E. coli* isolates were presumptively identified based on biochemical characteristics (Quinn *et al.,* 2004).

All the presumptive *E. coli* isolates were serotyped based on their somatic (O) antigen at National Salmonella and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh (India) and were also screened for STEC and ETEC pathotypes by PCR assay by detection of virulence genes of STEC (*stx1*, *stx2*, *eaeA* and *hlyA*) and ETEC (*LTA* and *ST1*) as described by Paton and Paton (1998) and Phipps *et al.* (1995), respectively (Table 1 and 2). The bacterial DNA lysate was prepared by boiling and snap chilling method.

RESULTS AND DISCUSSION

Escherichia coli count in retail meat

A proportion of 65.56 per cent beef and 58.88 per cent chicken samples exceeded the maximum permissible limit of ECC as per food safety and standards regulations (FSSAI, 2010) with the mean value of 3.07±0.2 $\log_{10}$ (cfu/g) and 2.98 ±0.03 $\log_{10}$ (cfu/g), respectively indicating high microbial contamination of raw meat. The finding was in close agreement with Ahmed *et al.* (2013) (2.94 $\log_{10}$ cfu/g) from Lahore, Pakistan. Heetun *et al.* (2015) and Huges *et al.* (2015) reported 5.1 $\log_{10}$ cfu/g and 4.80 $\log_{10}$ cfu/g *E. coli* from beef in Mauritus and Accra region, Ghana, respectively.

Prevalence of *E. coli* in retail meat

Overall 83.33 per cent and 80.00 per cent prevalence of *E. coli* strains in retail beef and, respectively were detected by *16S-r RNA* gene analysis from 3 districts of Mizoram. The prevalence of *E. coli* was significantly (P≤0.01) higher in chicken from Aizawl and Kolasib than Champhai district (Table 3). Saikia and Joshi (2012), Ahmed *et al.* (2013) and

Table 1: Oligonucleotide primers used for determination of virulence genes of *E. coli.*

Target gene	Primer name	Sequences (5′-3′)	Amplicon size (bp)	References
eaeA	*eaeA*-F	GACCCGGCACAAGCATAAGC	384	Paton and Paton
	eaeA-R	CCACCTGCAGCAACAAGAGG		(1998)
hlyA	*hlyA*-F	GCATCATCAAGCGTACGTTCC	534	Paton and Paton
	hlyA-R	AATGAGCCAAGCTGGTTAGCT		(1998)
stx1	*stx1*-F	ATAAATCGCCATTCGTTGACTAC	180	Paton and Paton
	stx1-R	AGAACGCCCACTGAGATCATC		(1998)
stx2	*stx2*-F	GGCACTGTCTGAAACTGCTCC	253	Paton and Paton
	stx2-R	TCGCCAGTTAATCTGACATTCTG		(1998)
LTA	*LTA*-F	GGCGACAGATTATACCGTGC	450	Phipps *et al.*
	LTA-R	CGGTCTCTATATTCCCTGTT		(1995)
ST1	*ST1*-F	ATTTTTCTTTCTGTATTGTCTT	190	Phipps *et al.*
	ST1-R	CACCCGGTACAGGCAGGATT		(1995)

Table 2: PCR thermal cycling conditions for virulence genes of STEC (*stx1, stx2, eae A* and *hly A*) and ETEC (*LT1* and *STA*).

Stages of PCR	Temperature/time		
	stx1 and *stx2*	*eae A* and *hly A*	*LT 1* and *ST A*
Initial heating	-	-	50°C for 5 minutes
Initial denaturation	95°C for 5 minutes	95°C for 5 minutes	95°C for 5 minutes
Denaturation	95°C for 45 seconds	95°C for 45 seconds	95°C for 45 seconds
Annealing	57°C for 45 seconds	65°C for 45 seconds	50°C for 45 seconds
Extension	72°C for 45 seconds	72°C for 45 seconds	72°C for 45 seconds
Final extension	72°C for 10 minutes	72°C for 5 minutes	72°C for 5 minutes
No of cycles	30	35	40

Ramya *et al.* (2015) also reported high prevalence of *E. coli* with 98, 54 and 76 per cent in chicken meat from retail market of North- East region (India), Lahore (Pakistan) and Bangalore (India), respectively. It indicated the contamination of retail beef and chicken might be due to unhygienic handling of raw meats during slaughtering, butchering and processing, improper storage, long exposure in the market environment and contamination from the handlers itself.

Serotypes of *E. coli* strains

The distribution of serotypes in 75 *E. coli* strains of beef showed that O118 (13.33%) was the most pre dominant serotype whereas the serotype O8 (13.89%) was most common in 72 *E. coli* strains from chicken followed by other serotypes (Fig 1 and 2). The serotypes O118 and O8 are frequently isolated from the potentially pathogenic STEC and ETEC pathotypes. The sero-groups O26, O45, O103, O111, O121 and O145 are the most commonly found non-O157 STEC strains (Gonzalez and Cerqueira, 2019). Detection of O118, O26, O111, O121 and O128 indicates the possible chance of transmitting the zoonotic STEC from retail meat to human. The most common ETEC serotypes associated with diarrhoea are O6, O8, O25, O78, O148 and O153 (Croxen *et al.,* 2013). Rathore *et al*. (2010) reported O8 and O9 as the most frequent serotypes from raw meat and meat products collected from Uttar Pradesh, India. Hazarika *et al.* (2007) had reported O145 as the predominant serotype in raw beef from retail shops of Assam, India. Jana and Mondal (2013) reported O120 as the most predominant serotype of *E. coli* isolated from raw poultry meat in West Bengal. *E. coli* serotypes O2, O20, O22 and O102 were

Table 3: Prevalence of *E. coli* in retail meats from 3 districts of Mizoram.

Sample	Aizawl	Kolasib	Champhai	Over all
Beef	25 (83.33)	26 (86.67)	24 (80.00)	75 (83.33)^{NS}
Chicken	26 (86.67)^{a}	27 (90.00)^{a}	19 (63.33)^{b}	72 (80.00)**
Total	51 (85.00)	53 (88.33)	43 (71.67)	147 (81.67)

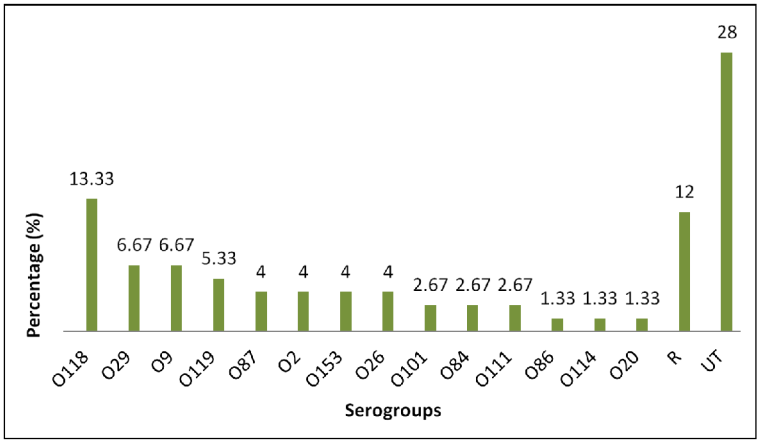

Fig 1: Distribution of *E. coli* serogroups in beef.

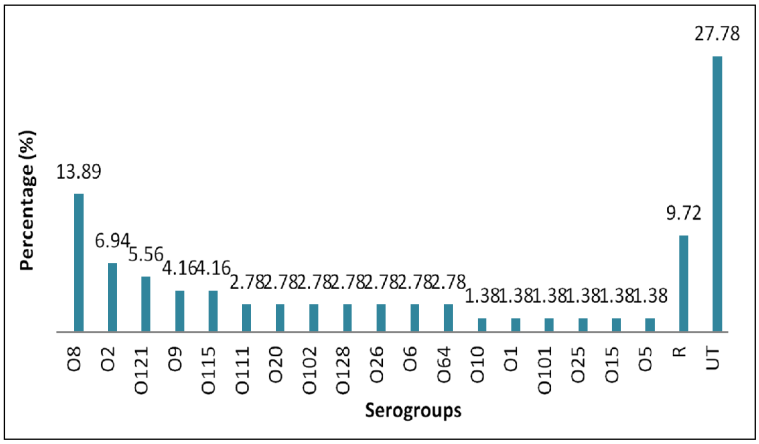

Fig 2: Distribution of *E. coli* serogroups in chicken meat.

reported from chicken meat in Mumbai (Zende *et al.,* 2013). Pratik *et al.* (2020) reported the serotypes O8, O35, O83, O88, O119 and O149 in chicken from Gujarat, India.

Detection of STEC genes

The prevalence of STEC in beef and chicken was recorded as 8.00% and 6.94 per cent, respectively with over all prevalence of 7.48 per cent (Fig 3). The prevalence of STEC in beef was higher than that of chicken meat which might be due to the fact that cattle are the most important reservoir of STEC contaminating environment, animal origin food and vegetables besides sheep and goat (Gonzalez and Cerqueira, 2019). Rashid *et al.* (2013) and Rasheed *et al.* (2014) had reported 25 per cent and 10.70 per cent of STEC from raw beef in Hyderabad and Jammu, India, respectively. Dutta *et al.* (2011) recorded 14.00 per cent STEC in chicken faeces from Mizoram, India.

The prevalence of *stx2* (6.67% and 4.16%) gene in *E. coli* strains was higher than the *stx1* (1.33% and 2.78%) in both beef and chicken, respectively (Fig 4, 5 and 6). However, 2.04 per cent *E. coli* strains from beef and chicken possessed both *stx1* and *stx2* genes. Hazarika *et al.* (2007), Aradhye *et al.* (2014) and Mohammed *et al.* (2014) also reported higher prevalence of *stx2* gene than *stx1* from beef in Assam, Mumbai and Pune, India, respectively. Zende *et al.* (2013) had reported 27.27 per cent *stx2* gene with 4.54 per cent prevalence of STEC in chicken from Mumbai but Saikia and Joshi (2012), Kiranmayi *et al.* (2011) and Rasheed *et al.* (2014) recorded higher prevalence of *stx1* gene than *stx2* in *E. coli* strains from chicken of North-East region, Hyderabad and Jammu region, India, respectively. Shiga toxin producing *stx2* gene is considered to be the most important virulence factor associated with *E. coli* infection in human. The predominance of *stx2* gene either alone or in combination with *stx1* has been found to be highly associated with HUS. The presence of STEC genes in *E. coli* in retail meat indicated the possibility of transmission of such organisms to human beings through food chain.

Higher prevalence of *eae A* gene in *E. coli* strains was recorded in beef (14.66%) than chicken (9.72%). Saikia and Joshi (2012) reported much higher prevalence (50%) of *eae A* gene in *E. coli* strains from retail chicken from North-East region, India. The *eae A* gene is an important factor for attachment and effacing lesion in human intestinal epithelial cells. Although *eae A* gene simultaneously occurs with *stx* gene, it is not essential required for STEC pathogenicity

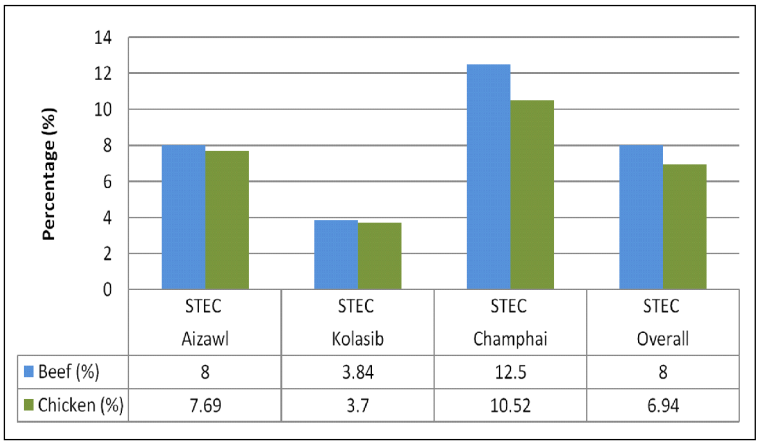

Fig 3: Distribution of STEC in beef and chicken meat from 3 districts of Mizoram.

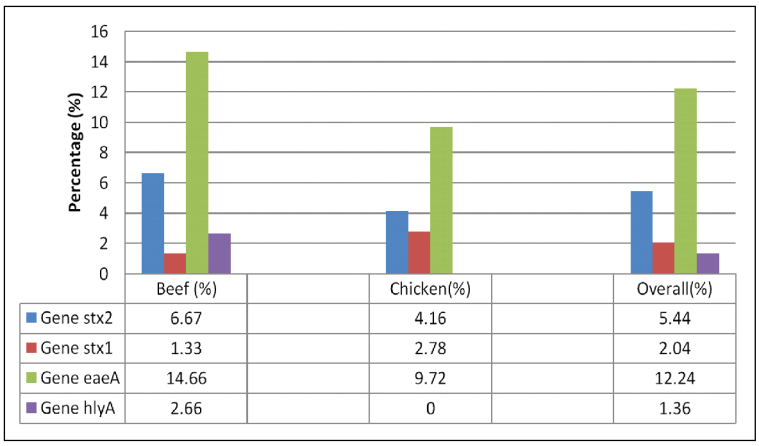

Fig 4: Distribution of *stx1, stx2* of STEC and associated *eaeA* and *hlyA* genes in *E. coli* isolates from beef and chicken meat.

(Gonzalez and Cerqueira, 2019) and *eae A* positive STEC occurs in both diarrhoeic and non-diarrheic animals (Coura *et al*., 2017) reinforcing that only some strains are able to cause disease. The occurrence of *hly A* gene was low in beef *E. coli* strains, 2.36 per cent singly and 2.04 per cent in combination of *eaeA* and *hlyA* and absent in chicken *E. coli* strains. Kiranmayi *et al*. (2011) and Rasheed *et al*. (2014) observed 10.50 per cent and 2 per cent *hlyA* gene in *E. coli* from retail meat of Hyderabad and Jammu region, respectively.

One beef *E. coli* strain was found to carry both the *stx2* and *hlyA* genes which belonged to the serotype O111. A virulent *E. coli* strains harboring both *stx* and *hlyA* genes may pose a higher risk to human health (Manna *et al.,* 2006). Serotype O26 and O111, one each from beef and chicken harbouring *stx2* gene has been frequently encountered with HC and HUS in human.

Detection of ETEC genes

The prevalence of ETEC strains was higher in chicken (26.38%) than beef (12.00%) with highest occurrence from Champhai district (31.57%) (Fig 7 and 8). The prevalence of *ST1* and *LTA* genes in chicken (15. 27% and 11.11%)

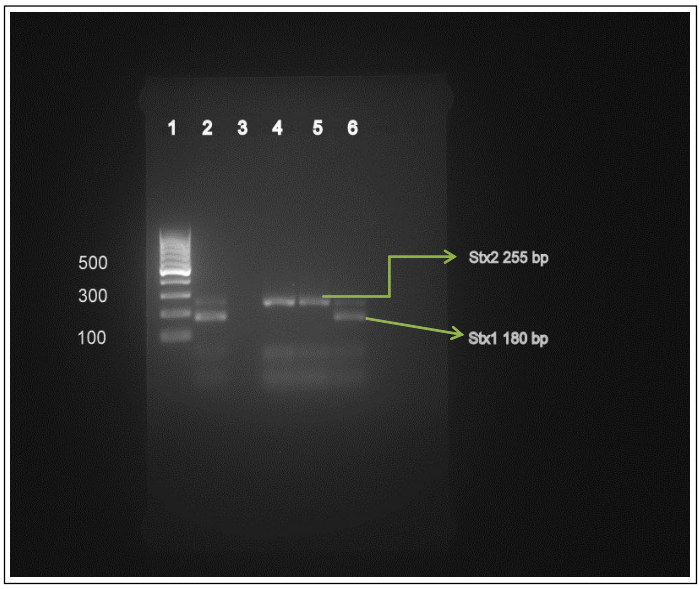

Fig 5: PCR amplification products of *stx1* (180 bp) and *stx2* (255 bp) genes of *E. coli.*
(L1: 100 bp DNA ladder, L2: Positive control of *stx1* and *stx2,* L3: No template control, L 4, 5: Positive isolate for *stx2*, L6: Positive isolate for *stx1* and *stx2*).

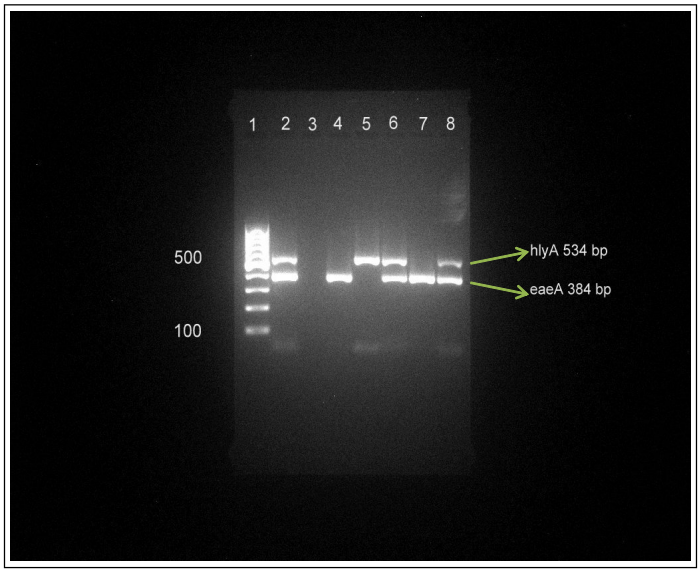

Fig 6: PCR amplification products of *eaeA* (384 bp) and *hlyA* (534 bp) genes of *E. coli.*
(L1: 100 bp DNA ladder, L2: Positive control of *eaeA* and *hlyA,* L3: No template control, L4 and 7: Positive isolates for *eaeA*, L5: Positive isolate for *hlyA,* L6 and 8: Positive isolates for both *eaeA* and *hlyA*).

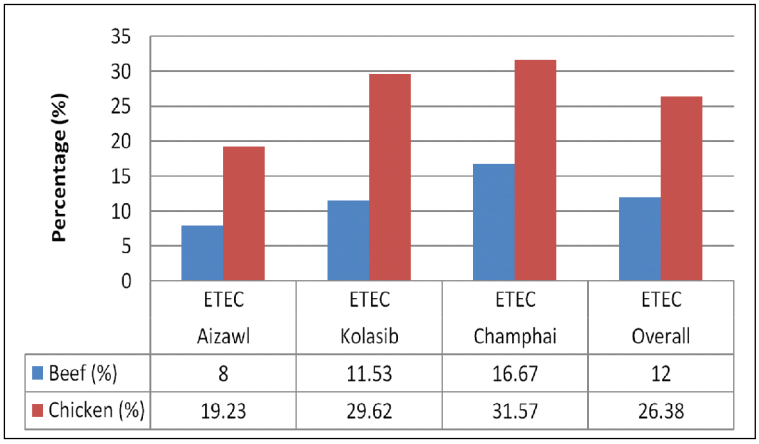

Fig 7: Distribution of ETEC in *E. coli* isolates of beef and chicken meat from 3 districts of Mizoram.

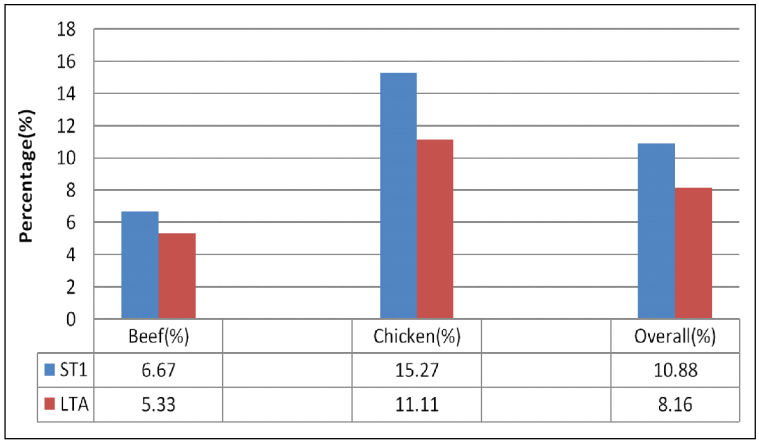

Fig 8: Distribution of *ST1* and *LTA* virulence genes in *E. coli* isolates from beef and chicken.

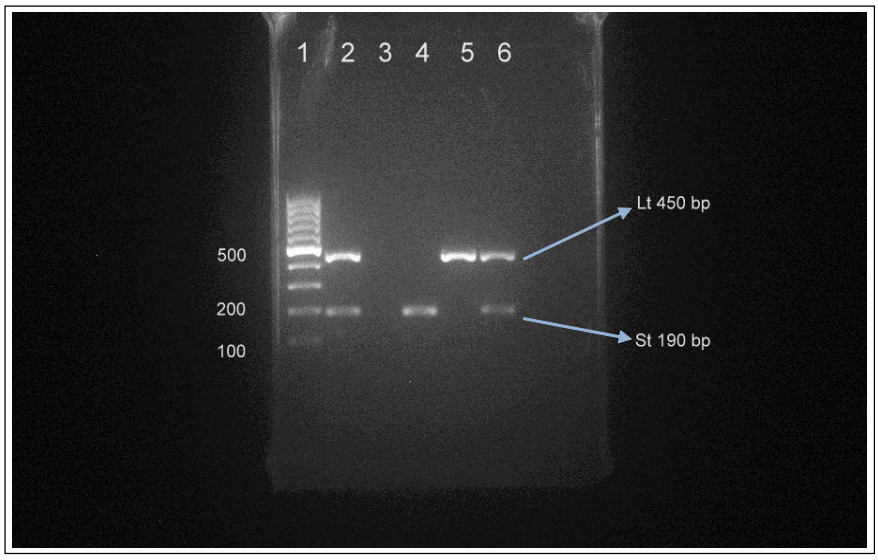

Fig 9: PCR amplification products of *ST1* (190 bp) and *LTA* (450 bp) genes of *E. coli.*
(L1: 100 bp DNA ladder, L2: Positive control of *ST1* and *LTA,* L3: No template control, L4: Positive isolate for *ST1*, L5: Positive isolate for *LTA,* L6: Positive isolate for both *ST1* and *LTA*).

was higher than beef (6.67% and 5.33%), respectively (Fig 9). Although ETEC infections are well studied in human, there is paucity of information on the occurrence of ETEC in meat from India. Zende *et al*. (2013) observed 18.28 per cent *ST1* gene and absence of *LTA* gene in *E. coli* from chicken in Mumbai. Yadav *et al*. (2007) detected 6.67 per cent *ST1* and 26.67 per cent *LTA* gene in *E. coli* strains from mutton in Madhya Pradesh. Few reports are also available on detection of ETEC in mithun (Rajkhowa *et al.,* 2009) and diarrhoeic lambs (Bandyopadhyay *et al.,* 2011) from North East India. Similarly, Mahanti *et al*. (2014) detected ETEC with *ST1* gene from healthy water buffalo in West Bengal.

CONCLUSION

The prevalence of pathogenic *E. coli* in meat may vary in different geographical locations. However, the paucity of studies makes it difficult to evaluate the actual role of retail meat as a STEC and ETEC vehicle in Mizoram. Detection of these two potentially pathogenic *E. coli* pathotypes in retailed beef and chicken bears public health significance owing to the fact that contaminated meat might act as vehicle for transferring pathogenic *E. coli* to human. Further, detection of potentially pathogenic *E. coli* serotype O26 and O111 harbouring *stx2* in retail meat of Mizoram are common serotypes associated with HC and HUS in human.

Conflict of interest: None.

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