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Isolation, Identification and Characterization of Escherichia Coli from Raw Meat, Fruit and Milk Samples of Local Area of Silchar, India

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Abstract: *Escherichia. coli* plays an important role in maintaining intestinal physiology. However, there are pathogenic strains that cause distinct syndromes of diarrhoeal disease. In this study a total of 80 raw samples (39=raw fruit, 25= raw milk and 16= raw meat samples) were collected from the local areas of Silchar to perform a detailed analysis of the molecular epidemiology of O157 strains by using PCR. Total 24 samples were confirmed to be *E. coli* .The samples were highly susceptible to Nalidixic Acid (83.4%) followed by Pipercillin (75%) and Levofloxacin (70.8%). Prevalence of EHEC was found to be 10.25% in fruit, 12% in milk and 18.75% in meat sample. The isolates showed a degree of diversity in PCR of stx1, *stx2* and *eaeA.* 3 strains had *stx1* gene, 4 strains had *stx2* genes and no strains were found to contain *eaeA* gene. PCR screening revealed the presence of toxin 3 (7.69%) in fruit samples, 2 (8%) in milk samples and 2 (12.5%) in meat samples.

Keywords: EHEC, Sorbitol non Fermentor, PCR, *stx1, stx2, eaeA.*

INTRODUCTION

Escherichia coli is a gram-negative, rod-shaped and facultative anaerobic bacterium that belongs to *Enterobacteriaceae* family. *Escherichia coli*is considered as an important pathogen of animals, comprising the normal flora of the gastrointestinal tract and the cause of septicemic disease in foals, calves, piglets and lamb. Most *E. coli* strains do not cause disease in human but certain types may cause serious illness and death. Shiga toxigenic *E. coli* (STEC) enteritis and hemolytic uremic syndrome (HUS) are connected with major mortality and morbidity, particularly among patients with severe renal and neurological disorders. Since the initial identification of *E. coli* O157:H7 as the etiological agent of HUS and Hemolytic colitis (HC) this pathogen has risen as amajor concern of food safety in many countries. The pathogencity of verotoxigenic *E.coli*(VTEC) is associated with a number of virulence factors such as Vero toxins (VT1and VT2), *eae*gene that encodes intimin which is responsible for attaching and effacing the organism to the gut epithelial cells lead to hemolytic uremic syndrome.

The aim of this study is to estimate the presence of Shiga toxin producing *Escherichia coli* from raw meat, milk and fruit samples. To perform antimicrobial susceptibility test of the bacterial isolates. Genotypic characterization of the isolates to check the presence of *stx1, stx2* and *eaeA* gene by Polymerase Chain Reaction (PCR) and gel electrophoresis.

MATERIALS AND METHODS

A total of 80 samples i.e. 39 raw fruit, 25 raw milk and 16 raw meat samples were randomly collected from local areas of Silchar in sterile bags. After performing serial dilution the samples were incubated in peptone water for 18- 24 hours at 37° C. The samples which showed turbidity in peptone water were streaked on MacConkey agar, dark pink colour colonies were picked up and streaked on selective media i.e. EMB agar, the green metallic sheen colonies were considered to be *E. coli.* Gram staining and motility tests were performed. The green metallic colonies were bichemically tested and the tests include Indole, MR-VP, Citrate utilization, Urease, Triple Sugar Iron and Carbohydrate fermentation test. After this the confirmed isolates were streaked on Sorbitol MacConkey Agar for distinguishing EHEC from nonpathogenic *E. coli*, the colourless(Sorbitol non fermenting) colonies were considered to be pathogenic strains and were stored at 4°C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed by the Kirby–Bauer disc diffusion method using Mueller– Hinton agar (HiMedia Laboratories, Mumbai, India), according to the Clinical and Laboratory Standards Institute guidelines. After incubating the inoculated plate aerobically at 37°C for 18 to 24 hrs in an aerobic atmosphere, the susceptibility of the *E. coli* isolates to each antimicrobial agent were measured and the results were interpreted in accordance

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with interpretive criteria provided by Clinical and Laboratory Standards Institute (2006).The antibiotics used in this study are - NalidixicAcid, Pipercillin/Tazobactum, Levofloxacin, Amoxyclav, Cefuroxime, Imipenem, and Kanamycin.

DNA Isolation

DNA isolation was done by boiling method. The desired stored isolates were inoculated in Nutrient agar and incubated at 37°C for 24 hours to obtain young culture prior to the extraction of genomic DNA. Then the colonies formed were scrapped into a 1.5 ml eppendroff tube containing 500µl PBS (pH 7.4).After that it is placed in a water bath for 20 minutes at 80°C.Centrifuged at 10000 rpm for 10 minutes. The supernatant so formed contains DNA was taken out in an eppendroff tube and preserved at -20°C.

Table 1: Oligonucleotide primers used for amplification of toxin genes

Primer	Sequence (5' to 3')	Expected Amplicon size	References
stx1 F	Forward:ACACTGGATGATCTCAGTGG	655	(Paddock *et al.*, 2013)
stx1 R	Reverse:CTGAATCCCCCTCCATTATG		
stx2 F	Forward: CCATGACAACGGACAGCAGTT	779	(Ashraf *et al.*, 2015)
stx2 R	Reverse: CCTGTCAACTGAGCAGCACTTTG		
eaeA F	Forward : GACCCGGCACAAGCATAAGC	384	(Jakee*et al.*, 2012)
eaeA R	Reverse:CCACCTGCAGCAACAAGAGG		

PCR Experiment

The genomic DNA from every isolates of *E. coli* was used as template for PCR experiments. The sequences of the nucleotides primers studied in this research are shown in Table 1. PCR reaction mixture: Each single reaction mixture 25µl contained 2.5µl of Taq buffer (1X), 0.5 µl dNTPs(200µm), 1U of Taq DNA Polymerase (Qiagen, Mumbai, India), 1µl of each primer (10 picomole) and 2µl of template DNA. Nuclease free water added to make the final volume up to 25µl. The generated amplicons were visualized by electrophoresis on 0.8% agarose gel stained with ethidium bromide and visualized under Gel Doc EZ imager (Bio-Rad).

Table 2: PCR condition

GENE	REACTION CONDITION	
stx1	Initial denaturation at 95°C for 2minutes.	
	Denaturation at 95°C for 30 seconds.	
	Annealing at 58° C for 30 seconds.	35 cycles
	Extension at 72°C for 40 seconds.	
	Final extension at 72° C for 10 minutes	
stx2	nitial denaturation at 94°C for 5minutes.	
	Denaturation at 94°C for 30 seconds.	
	Annealing at 59°C for 30 seconds	32 cycles
	Extension at 72°C for 90 seconds.	
	Final extension at 72° C for 10 minutes.	
eaeA	Initial denaturation at 94°C for 5minutes.	
	Denaturation at 94°C for 1 minute.	
	Annealing at 58°C for 30 seconds.	32 cycles
	Extension at 72°C for 45 seconds.	
	Final extension at 72°C for 8 minutes.	

RESULT AND DISCUSSION

Out of 80 samples i.e. 39 fruit, 25 milk and 16 meat samples 24 isolates were confirmed in the study as *E. coli* by culturing in selective media by performing gram staining, motility test and IMViC test. The occurrence of *E.coli* in the fruit was 35.9% i.e. 14 sample, 24% in milk i.e. 6 isolates and 25% in milk i.e. 4 isolates.

The total prevalence of *E. coli* O157:H7 was 41.6% i.e. 10 isolates out of 24 *E. coli* isolates.10.25% (4/39 samples) in fruit, 12% (3/25 samples) in milk and 18.75% (3/16 samples) in meat samples.

Cattles are the principal reservoir of *E. coli* O157:H7, which is frequently excreted in their feces and responsible for an increasing number of food based infection, Milk can be easily mixture by infected food handlers who do bad individual hygiene or by water lodged containing human wastes. Poor personal hygienic practice and related factors, these may be because fruit handlers do not use glove, hair nits and different fruit buyers touched by their hands for sorting healthy fruits during marketing. Meats are often split into pieces to meet customer's financial affordability. The cut surfaces in most cases are not covered to

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protect them from contaminants in the surrounding environments thus they may be easily contaminated by air-borne pathogens.

In this present study antimicrobial susceptibility pattern against 7 antibiotics were studied for 24 *E. coli* isolates. The result was interpreted according to the diameter of the zone of inhibition as per the manufacturer's HIMEDIA, MUMBAI, India instructions. The bacterial isolates were found highly susceptible against Nalidixic acid (83.4%) followed by Pipercillin (75%), Levofloxacin (70.8%) and highly resistant against Cefuroxime (66.6%) followed by Amoxyclav (54%). The frequencies of bacterial strains resistant to antimicrobial agents have increased dramatically in the environment as consequences of the widespread use of antimicrobial drugs (Kurse & sorum, 1994).

In this study PCR based method for detection of *stx1*, *stx2* and *eaeA* genes used to access virulence potential of the isolates. PCR screening revealed the presence of toxin 3 (7.69%) in fruit samples, 2 (8%) in milk samples and 2 (12.5%)in meat samples. Similar trend in regard to *stx1* and *stx2*genes was observed in study of Al-Kharousi *et al*. 2016 (in case of fruit), Al-Zogibi *et al*. 2015 (in case of milk) and Abdaslam *et al*. 2014 (in case of meat).In this present study 2(8%)milk sample,3 (7.69%)fruit sample,2 (12.5%) meat sample out of 25,39 and 16 samples showed the presence of toxin genes. However, Al-Kharousi *et al*. 2016 obtained 20% result out of 105 fruit and vegetable samples, Al-Zogibi *et al*.,2015 obtained 4.81% toxin isolates out of 540 milk samples and Abdaslam *et al*. (2014) obtained 11.1% toxin isolates out of 90 meat samples.

This study revealed that culturable EHEC (*E. coli* O157:H7) were present in raw fruit, milk and meat samples examined from local markets in Silchar, India with the occurrence of 7.69 %(fruit), 8% (milk) and 12.5% (meat). The low occurrence of *E. coli* O157:H7 in the fruit, milk and meat samples observed in this study is very much agreement with previous studies in other parts of the world (Abu-Duhier *et al.*, 2015; Lambertini *et al*., 2013; Akkaya *et al.*, 2006), who reported the occurrence rates of 14.3%,35% and 1.05% respectively. The result for *eaeA* toxin is negative which indicate that either that these toxin is absent from the samples that were studied or that they are present in very low counts. In 2003 and 2004, Mukherjee *et al.* 2004 in a microbiological survey of fresh produce with *E. coli* O157:H7 being one of their target pathogenic bacteria failed to isolate the bacteria from the fruits and vegetables.

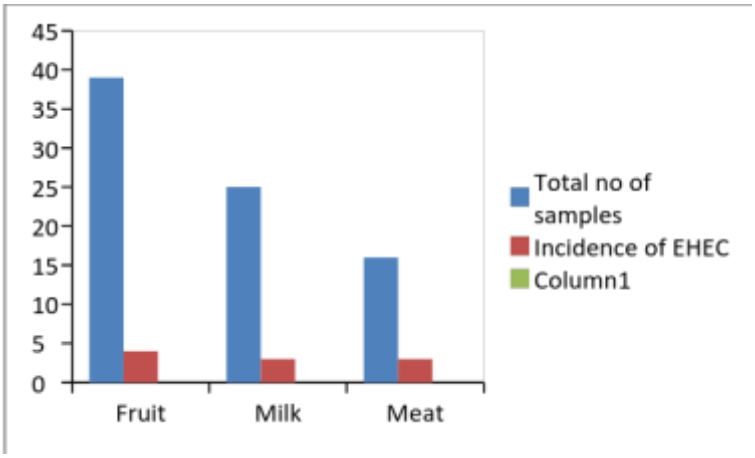

Figure 1. Graphical representation of incidence of EHEC (EnteroHaemorrhagic*E. coli*)

Table 3: Frequency of distribution of *eaeA, stx1 and stx2* genes in *E. coli* isolates.

Sl. No	Sample of Material	No. of Samples	Incidence of toxins (%)	*stx1*	*stx2*	*eaeA*
1	Fruit	39	3(7.69%)	2	-	-
2	Milk	25	2(8%)	1	2	-
3	Meat	16	2(12.5%)	-	2	-
Total		80	7(8.75%)	3	4	-

CONCLUSION

The members of *E. coli* are widely distributed in the environment. The bacterium spreads by contaminated food and water. Most *E. coli* is normal flora found in intestinal tract. Possession of virulence factors such as exotoxin distinguishes the normal flora and pathogenic strains of the organism. Meat, fresh produce such as raw vegetables and fruits, unpasteurized milk, water supplies are the sources of *E. coli* contamination in food business and also by direct contact between raw foods and ready –to-eat foods and poor personal hygiene practices. Risk of cross contamination in food business increases through clothing, aprons and gloves. Adhering to strict food

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safety management procedures in all areas involving the storing and handling of foods including surfaces, equipment and personal hygiene of staff can only prevent the spread of this contamination. Complete enteric disease inspection strategies, prevention and education are necessary for meeting the challenges in the years ahead. The issue of surveillance must be among our highest priorities and understanding the role of food borne disease plays as an emerging infectious problem in the country. Raw meat get contaminated by pathogenic *E. coli* strains and represents a public health hazard, therefore measures should be taken to avoid such contamination, particularly through cross contamination in locals. Contamination of fruit, milk and meat with *E. coli* O157:H7 as was revealed in this research make stronger the require of surveillance for *E. coli* O157:H7. Consumers should be educated about the potential risk of consuming unhygienic fruit, improperly cooked meat and milk. Regulatory and educational efforts are needed to improve the safety of milk, meat and fresh farm fruits.

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