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CULTURE-INDEPENDENT PCR DETECTION OF ENTEROTOXIGENIC *Escherichia coli* IN STREET VENDED FRESH FRUIT JUICES IN HOMA BAY TOWN, KENYA

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Abstract: **Fresh fruit juices are well recognized for their nutritive value. In many tropical countries they are common man's beverages and are sold at all public places including along the streets. Although they are healthy and nutritious, the methods used for their extraction are unhygienic and the utensils are quickly and carelessly cleaned thereby causing contamination by disease causing microbes, resulting in food borne diseases. Enterotoxigenic Escherichia coli is one such bacterial foodborne pathogens of significance. It is the leading cause of diarrhea in the developing world where there is inadequate clean water and poor sanitation. ETEC diarrhea occurs in all age groups, but mortality is most common in infants, particularly in the most undernourished or malnourished infants in developing nations. It is also the most frequent bacterial cause of diarrhea in traveler's. ETEC infection occurs through consumption of contaminated food or water. Rapid and specific detection of enterotoxigenic Escherichia coli (ETEC) in food is critical for the management of the food-borne diarrheal diseases threatening human lives. Conventional methods for detection of ETEC involve enrichment and biochemical identification which are time consuming and less sensitive. Polymerase Chain Reaction, using specific primers is a powerful molecular technique for rapid and accurate detection of ETEC. The present study aims to detect ETEC bearing virulent signature gene LT1 in food samples. Street sold fruit juices were purchased from the venders in the streets of Homa bay town. Multigenomic DNA templates were prepared by boiling prep and purified using Sodium acetate-ethanol method. This was followed by PCR targeting the LT1 gene to detect ETEC in food samples. The reference strains (Enterotoxigenic *Escherichia coli* MTCC 723) was used as positive control. Fresh fruit juice samples were analyzed and found contaminated with ETEC. The contaminats' DNA amplification based assay used here is rapid with high specificity for detection of ETEC in food samples.**

Keywords: **Enterotoxigenic Escherichia coli, PCR, Food samples.**

1. INTRODUCTION

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. In many tropical countries they are common man's beverages and are sold at all public places and roadside shops. Although they are healthy and nutritious, the methods used for their extraction are not hygienic and the utensils are cleaned quickly and carelessly, so they pose a health risk due to contamination with various disease causing microorganisms (Lewis *et al*, 2004). The various sources of contamination include environmental exposure, use of unhygienic water for dilution, Improper washing of fruits, dressing

with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust (Buck*et al.,* 2002; **Kaur and Mehdi, 2015**). One such disease causing microorganism is Enterotoxigenic Escherichia coli (ETEC). Enterotoxigenic *Escherichia coli* (ETEC) was first recognized as a cause of human illness in the 1960s (Sack *et al.,* 1971) To date, is regarded as a major cause of *E. coli* mediated diarrhea in humans, affecting mainly children and travelers (Åsa Sjöling *et al*., 20150). In 2010 alone, 28.7 million episodes have been reported due to ETEC and 45,713 deaths in WHO regions of South Asia (Lamberti *et al.,*2014). Contaminated water and food have been implicated as vehicles for transmission of ETEC infection in humans (Qadri *et al.,* 2005). A number of food matrices which include fresh fruit juices are the potential carriers of ETEC. ETEC infections, reported are always associated with poor hygiene and sanitation (Tomar *et al.,* 2014). ETEC secretes two types of enterotoxins (heat-labile, LT; and heat-stable, ST enterotoxins) encoded by LT1 and ST1 genes, (Turner *et al.,* 2006). The heat-labile enterotoxins are classified into two major groups (LTI and LTII). LTI is expressed by E. coli strains that are pathogenic for both human and animals. The LT1 gene commonly present in strains associated with human.

Escherichia coli (ETEC is difficult to detect and isolate. The conventional methods for its detection and quantitative enumeration include cell culture techniques, enzyme linked immunosorbent assays, and membrane-based DNA hybridization assays (Turner *et al.,* 2010). All these methods are labour intensive and time consuming (Khan *et al.,* 2007; Lijima et al 2007 Currently, Polymerase Chain Reaction technique is regarded as a powerful and highly specific technology which allows amplification and detection of target. Studies have reported the presence of ETEC in water and other types of food items sold in streets all over the world.(Ram et al 2008; **Kaur and Mehdi, 2015**).

Homa bay town, like many other towns all over the world has a thriving business of selling food including fresh fruit juices along the streets. In this town, there is always a great demand for fresh juices as the climate remains hot for most part of the year and street vendors serve a great portion of this demand, but their hygiene practices is questionable (Schaffner *et al,,* 2005). The vended juices are extracted by squeezing manually or using juice extractors then homogenized using blender, and finally served after considerable dilution with water whose source remains unclear. Despite quality measures put in place by the department of public health of the Homa bay county government, there is still sporadic cases of outbreaks of gastroenteritis caused by various pathogenic bacteria, ETEC included.

A study by Suneetha *et al* (2011) reported *E.coli, Salomonella, L.casei, L.acidophilus* species as the prominent microbial pathogens involved in unpasteurized juice contamination. Therefore this study analysed vended fresh fruit juices for contamination by Enterotoxigenic *Escherichia coli* through PCR technique.

2. MATERIALS AND METHODS

Juice Sampling:

300ml of Fresh fruit juice samples of Avocado, Pineapple and mango were procured from the street venders in Homa bay town. These were representative samples of those sold along the streets. The purchase of juice samples was done for five consecutive days and each time analysed. Fruit juices were filtered through sterilized muslin clothes to remove the coarse fibres. After this 100mls of the samples were concentrated to 500µl by centrifugation at 14000 x g.

Extraction of Multigenomic DNA:

DNA template was prepared by boiling the 500 µl concentrated juice sample and removing the debris by centrifugation at 16,000 x g for 5 min at 4°C. DNA was precipitated from supernatant using 0.3 M sodium acetate (pH 5.2) and ice cold ethanol (Ram et al., 2007). The precipitated DNA was pelleted by centrifugation at 12000 x g for 5 min. DNA pellet was washed thrice with 70% ethanol and finally dissolved in 250 µl TE (pH 8.0). The purity and yield of isolated DNA samples were determined by Spectrophotometer.

Detection of ETEC Using PCR:

PCR targeting the virulent LT1 gene was run to detect the ETEC in fruit juice samples. The reaction mixture in a final volume of 50 µl comprised of dNTPs (0.2 mM), Taq DNA polymerase (1.5 units), 10x reaction buffer (5 µl), MgCl2 (1.5 mM), primers (0.4 µM, each) for LT1 gene and DNA template (5 µl). The PCR program was as follows: initial denaturation at 950C for 3 min and then 45 cycles at 95 0C for 20 s, 55.8 0C for 30s, and 720C for 30s. **The** reference strains (Enterotoxigenic *Escherichia coli* MTCC 723) was used as positive control for PCR.

Gel electrophoresis:

Amplification products were subjected to horizontal gel electrophoresis in 1% agarose gel in TBE (Tris-borate EDTA) buffer at room temperature at 100 volt (50 mA) for 1h. Briefly, 10 µl of amplified DNA for each sample was mixed with 1 µl of tracking dye and loaded into an individual well of the gel (5 mm thick). DNA bands were detected by staining the gel with ethidium bromide (0.5 µg/ml) for 30 minutes at room temperature and photographs were taken according to the procedure. 100 bp DNA size standard (Invitrogen, USA) was used as marker to measure the molecular size of the amplified products.

Primers:

For specific detection of ETEC harboring LT1 gene in surface food samples, primers (F: 5′-GGCAGGCAAAAGA GAAATGG-3′ R: 5′-TTGGTCTCGGTCAGATATGTG-3′, position: 996- 1145, product size 150 bp) were adopted from Ram et al, (2008)

Table 1: Nucleotide sequences of candidate oligomers of LT1 gene of enterotoxigenic Escherichia coli.

Gene	Primer(5'-3')	**Product length (bp)**/position of primers
(Heat Labile Toxin) ***LT1***	GGCAGGCAAAAGAGAAATGG	**150**
	TTGGTCTCGGTCAGATATGTG	111-260

3. RESULTS

Specificity of the Assay:

The PCR reaction generated the product of 150 bp as evident on agarose gel electrophoresis. The PCR assay described here is highly specific to the ETEC. All strains of E. coli exhibiting LT1 gene were positive in PCR assay.

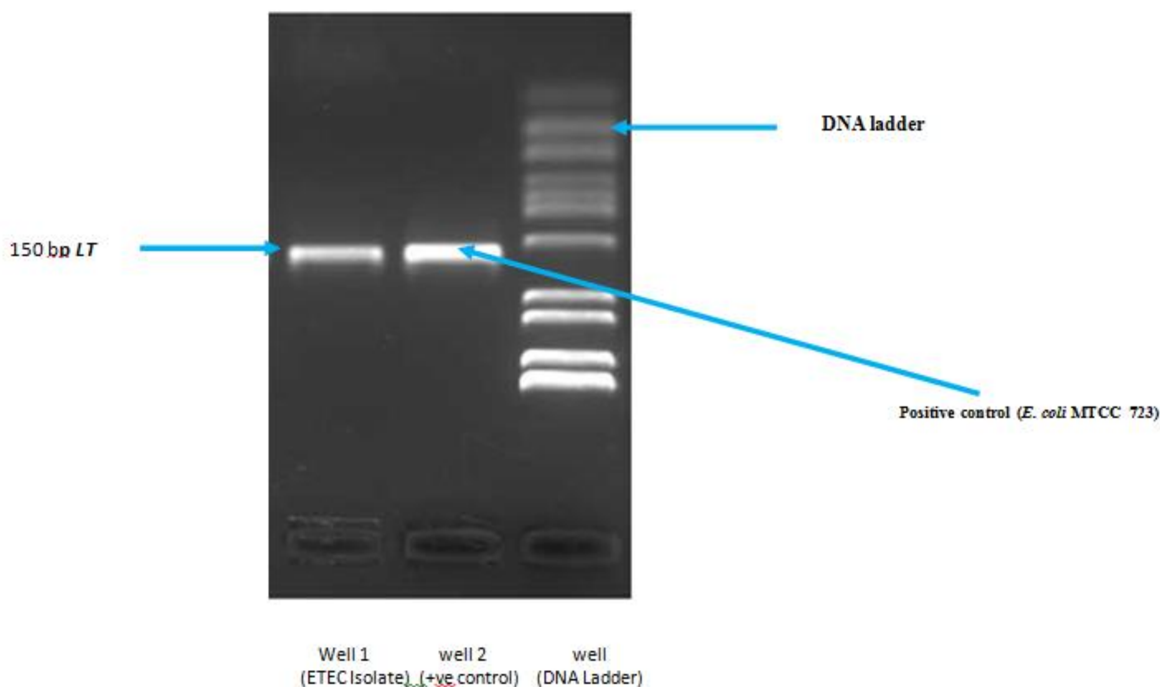

Fig 1: Agarose gel electrophoretic analysis of amplicon of LT gene

Culture-Independent PCR Detection of Etec in Fresh fruit Samples:

Fresh fruit juice samples purchase from the street vendors were analyzed for the presence of ETEC all the samples were found positive for the presence of ETEC.

Table 2: Contamination of street fruit juices by Enterotoxigenic Escherichia coli

	JUICE TYPE	ETEC
1.	AVOCADO FRUIT JUICE	+
2.	MANGO FRUIT JUICE	+
3.	PINEAPPLE FRUIT JUICE	+

4. DISCUSSION

The present study has opened a new avenue in the rapid and accurate culture independent detection of ETEC in food samples such as fruit juices. LT1 gene was targeted for the PCR assay. The primer pair was adopted from Ram et al, in which the limit of detection was 2 CFU (Ram et al 2007). Specificity of the assay was checked with strain of E. coli and other genus. The assay was negative for other bacterial strain lacking target gene. These observations validate the high specificity of the assay. Studies by Lothigius et al (2010) indicate that ETEC can survive for longer periods up to 3 months in fresh and sea water. This leads to the constant exposure and accumulation of ETEC in such waters which are continuously used by venders for either cleaning of utensils or dilution of the fruit juices during their preparations. In the present study, all the juice samples were found contaminated with ETEC. The present assay overcomes the limitations of conventional detection methods. The culture-based assays are lengthy, and are unable to detect viable but non-culturable state of ETEC (Lijima *et al., 2007*). Therefore, the culture independent PCR employed in this study is rapid procedure with high specificity detection of ETEC in food samples.

5. CONCLUSION

These findings demonstrate that street vended fruit juices sold in the street of Homa Bay town contain some pathogenic organisms which are likely to be a potential hazard to the health. In conclusion, results obtained in this study indicated that consuming of drinks/juices sold by mobile street-vendors, in many locations of Homa bay town may possess a serious challenge to the consumers.

6. RECOMMENDATIONS

The results of this study should push the concerned authorities to issue hygienic regulations that should be compulsory for all food vendors in town so as to control cases of microbial contaminations that may pose serious health challenges to the consumers.

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