

Detection of *stx* genes in *E. coli* samples isolated from pork and beef obtained from retail shops in Shillong, Meghalaya.



Microbiology

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ABSTRACT

Escherichia coli strains like O157:H7 produce Shiga toxins that cause gastrointestinal diseases in humans. In this study, 27 pork and 26 beef samples were obtained from retail shops in Shillong for isolation of *E. coli*. Pure cultures were isolated on EMB agar from 7 pork (35%) and 1 beef (8.33%). The isolates were then subjected to various biochemical tests which confirmed their identity. Multiplex PCR was used to amplify the genes *stx1* and *stx2* (Shiga toxin genes) after DNA extraction from the isolates. Amplification of *Stx* genes was seen in 2 (25%) of the pork isolates while no amplification was seen in the beef isolates. The results indicate that pork may be more contaminated by *stx* harbouring *E. coli* than beef.

INTRODUCTION

Escherichia coli was first described by Escherich in 1885. Since then *E. coli* has become the most studied and one of the most important bacterial pathogens. Like other members of the Enterobacteriaceae family, *E. coli* also has several serotypes. One serotype was found to harbour the Shiga toxin genes (*stx1* and *stx2*) that produce toxins (Stx₁ and Stx₂ respectively) similar to that produced by *Shigella dysenteriae* and is thus called Shiga-toxin-producing *Escherichia coli* (STEC). In the early 1980s, STEC were found to be involved in enteric diseases (Kehl, 2002). These organisms have been found to be the causes of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), two fatal food borne infections in man (Mora et al., 2007). The most usual pathway for entry of STEC in the body is by the consumption of contaminated meat and dairy products. The most likely reservoir of STEC is cattle. (Doyle & Schoeni, 1987; Grimm et al., 1995; Kudva, Hatfield & Hovde, 1997; Mora et al., 2007; Moses, Egwu & Ameh, 2006; Padhye & Doyle, 1991; Samadpour et al., 1994; Suthienkul et al., 1990; Wells et al., 1991). Most individuals infected with STEC are asymptomatic. Hence, there is a greater threat of the spread of infection by contact between such an individual and a healthy individual. Studies have also shown that most of the severe human illnesses are caused by strains of STEC that produce only Stx₂ (Paton & Paton, b. 1998). Several measures are therefore necessary to detect the presence of STEC on food stuffs. One such method is the Multiplex PCR which can detect both *stx* genes (Fratamico, Sackitey, Wiedmann & Deng, 1995; Khan et al., 2002; Kim et al., 2005; Paton & Paton, a. 1998). In this study, the presence of STEC in pork and beef samples sold in retail shops of Shillong, was determined by biochemical characterization and Multiplex PCR. The objective of this study is to highlight the dangers of STEC contamination of meat which is a commonly consumed food stuff in this North-eastern city of India.

MATERIALS AND METHODS

Sample collection

A total of 53 meat samples - 27 pork and 26 beef were collected from various retail shops in Shillong in sterile sample containers.

Enrichment procedure

The samples were inoculated in Mac Conkey broth (Sisco Research Lab, Mumbai, India) for enrichment of *E. coli* cultures and incubated at 37°C overnight.

Isolation of *E. coli*

After 24 hours, the enriched culture was then aseptically streaked on to freshly prepared, sterile Eosin Methylene Blue (EMB) agar plates (Hi Media, India) and incubated at 37°C for 24 hours to get single pure colonies with a metallic green sheen.

Biochemical characterization

The isolated colonies were confirmed to be that of *E. coli* by Gram staining, Indole, Methyl red, Voges-Proskauer and Citrate utilization tests (Kudva et al., 1997). Apart from these tests, other tests performed were oxidase test, catalase test, triple sugar iron (TSI) test and sugar fermentation tests where the ability of the bacteria to degrade adonitol, raffinose and cellobiose was determined.

DNA isolation

The isolated bacteria were incubated overnight in Brain Heart Infusion (BHI) Broth (Hi Media, India) at 37°C. The cultures were then centrifuged at 8000 rpm for 2 minutes. The supernatant was discarded. The pellet was resuspended in 100µl of autoclaved distilled water. The tubes were kept in boiling water for 10 – 15 minutes and then immediately put into ice. The supernatant contains the DNA template. (Kim et al., 2005)

Multiplex PCR

The presence of virulence genes *stx1* and *stx2* in the isolates was analysed by Multiplex PCR which allows the simultaneous amplification of both the genes in a single reaction by using two primer pairs. (Table 1).

Table 1. Primers used in the amplification of *stx1* and *stx2*.

Primer sequence	Target genes (size)	Reference
LP30 5'-CAGTTAATGTGGTGGCGAAGG-3' LP31 5'-CACCAGACAATGTAACCGCTG-3'	<i>stx1</i> (348bp)	Feng, Weagant & Monday, 2001
LP43 5'-ATCCTATTCCCGGGAGTTTACG-3' LP44 5'-GCGTCATCGTATACACAGGAGC-3'	<i>stx2</i> (584bp)	

PCR conditions consisted of an initial 94°C denaturation step for 5 mins followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 mins. The final extension cycle was followed at 72°C for 10 mins. Amplified DNA fragments were resolved by gel electrophoresis using 1.5% agarose gel in Tris-acetate-EDTA (TAE) buffer. Gel was stained with 0.5 µl of Ethidium bromide (EtBr) per ml, visualized and photographed under UV illumination.

RESULTS

Isolation of *E. coli*

Suspected *E. coli* colonies appeared on the EMB agar plates with a typical metallic sheen from only 32 (60.37%) samples - 20 pork (74.07%) and 12 beef (46.15%) samples.

Biochemical characterization

Gram staining revealed pure cultures in only 8 (25%) samples, 7

pork (35%) and 1 beef (8.33 %). The 8 isolates showed standard *E. coli* characteristics for the Indole, Methyl red, Voges-Proskauer, Citrate utilization, Oxidase and Catalase tests. The TSI test produced a variety of results depending on the fermentation of glucose, lactose or sucrose. No Hydrogen gas production was seen in this test. The sugar fermentation tests revealed only 1 (12.5%) of the isolates fermenting adonitol while raffinose and cellobiose were both fermented by 2 (25%) of the isolates each.

Detection of *stx* genes.

The results obtained from Multiplex PCR showed the presence of *stx*₁ genes (348 bp) in 2 (25%) of the 8 isolates while *stx*₂ (584 bp) was detected in only 1 (12.5%) isolate. The isolate containing *stx*₂ was also one of the 2 isolates containing *stx*₁. The other isolates showed no amplification or unspecific primer binding. Both the genes were detected from pork isolates.

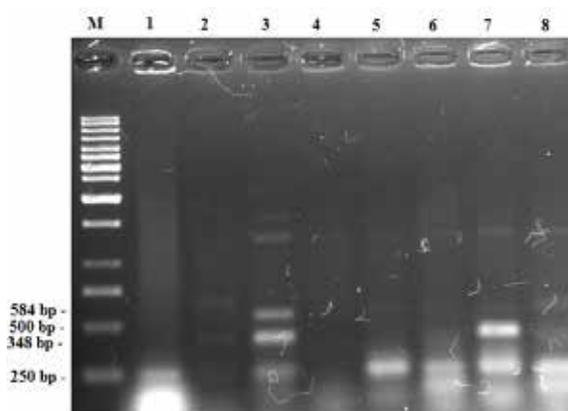


Fig.1. Multiplex PCR yielded amplification of *stx*₁ (348 bp) in lane 3 and 7 while *stx*₂ (584 bp) was detected in lane 3 only. M is a 250 bp DNA ladder. Lanes 1-7 are pork isolates while lane 8 is the beef isolate.

DISCUSSION

This study showed STEC contamination only in pork and not in beef even though studies have suggested cattle to be the main reservoirs of STEC strains (Kehl, 2002; Kim et al., 2005). This may be due to fecal contamination. Although the present study determined a very low percentage of STEC contaminated meat samples – 2 (3.77%) out of 53, the detection of the pathogen raises safety concerns regarding the possible spread infection among the human populace. One way of preventing STEC contamination is by the administration of antibiotics as most STEC strains are susceptible to many antibiotics. However, antibiotic resistant strains have also been isolated (Kim et al., 2005). It is thus advisable to limit the use of antibiotics as this could lead to the emergence of more resistant strains. The results obtained suggest that raw meat like pork is an important source of STEC. There is also the possibility of STEC contamination in other food stuffs (Doyle & Schoeni, 1987; Kudva et al., 1997; Padhye & Doyle, 1991; Samadpour et al., 1994). In any case, multiplex PCR has become one of the most concrete tool in the detection of STEC.

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