Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Detection of Toxin producing *E. Coli* Isolates from Panipuri sold in Bangalore sold in Bangalore by PCR Analysis

Mary C., Usha M. S.*
Department of Microbiology, Centre for P.G. Studies, 18/3, 9th Cross, 3rd Block, Jayanager, Jain University, Bangalore-11, Karnataka, India.

Received on: 01-11-2012; Revised and Accepted on: 14-11-2012

ABSTRACT

Out of 100 samples of panipuri collected 74 were positive for E. coli. Isolates pe16, pe68, pe74, pe88 and pe93 were selected based on their protein production and multi drug resistance properties. All the five isolates were subjected for subjected to cytotoxic effect and PCR analysis. β-haemolysis were shown by all the five isolates on micro-titre plates. PCR analysis of the isolates showed that all five are negative for Stx-1 whereas for Stx-2 only pe16 is positive and the rest four are negative.

Key words: *E. coli, panipuri, PCR analysis, toxin production.*

INTRODUCTION

E. coli is a consistent inhabitant of the human intestinal tract and it is the predominant facultative organism in the human gastrointestinal tract. *E. coli* is frequently contaminating organism and is a reliable indicator of fecal pollution, generally in insanitary conditions of water, food, milk and other dairy products. But some strains when they enter through digestive system they can cause illness, due to toxins they secrete. The toxins produced by *E. coli* strains include heat-labile or heat-stable enterotoxins and Shiga-like toxin. Shiga-like toxin, also known as verotoxin, is a toxin generated by some strains of *Escherichia coli*. It is named for its similarity to the AB-5 type Shiga toxin produced by the bacteria *Shigella dysenteriae*. There are two types, known as stx-1 and stx-2. Shiga toxin-producing *E. coli* is a type of enterohemorrhagic *E. coli* (EHEC) bacteria that cause illness ranging from mild intestinal disease to severe kidney complication. Shiga toxin producing *E. coli* causes a condition known as hemorrhagic colitis. Shiga toxin is also responsible for hemolytic uremic syndrome, a life threatening complication that causes kidney failure.

E. coli O157:H7 is a part of the enterohemorrhagic group of *E. coli*. This pathogen produces verotoxins that can cause thrombotic thrombocytopenic purpura, hemorrhagic colitis and hemolytic ureamic syndrome. Six genes of *E. coli* O157:H7 are generally targeted for PCR confirmation, namely *rfbE* (O157 antigen), *eae* (intimin), *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *hlyA* (hemolysin) and *fliCh7* (flagellar antigen) [1].

Study on toxins and genes involved in toxin production by *E. coli* have been carried out by few investigators. Honda *et al.* showed the use of Biken test in which a precipitin line will be formed in the agar between *E. coli* colony producing heat-labile (LT) enterotoxin and anti-cholera-toxin or anti-LT sera placed in an adjacent wall [2]. Shigella dysenteriae-1 like toxin produced by *E. coli* has been purified and characterized by O'Brien and Laveck.[3] Jeshveen *et al.* has worked on optimization of multiplex PCR conditions for rapid detection of *E. coli* O157:H7 virulence genes [4].

MATERIALS AND METHODS

Media and antibiotics:

Dehydrated media and other chemicals used for the present study were procured from Hi-media Pvt. Ltd., Bombay, India.

***Corresponding author:**
Usha M. S.
Lecturer,
Centre for Post Graduate Studies,
18/3, 9th Main, 3rd Block, Jayanagar, Jain University,
Bangalore-11. Ph. No. +91 9845342346, +91 80 41210691.
Fax. +91 80 41210692
**E-Mail: bg.ushams@gmail.com*

Isolation of E. coli:

A total of 100 samples were collected from street vendors in and around Bangalore using sterile containers. Isolation of *E. coli* was carried out using Eosin Methylene Blue agar media. One ml of each samples were used for isolation purpose. Nucleated colonies with or without metallic sheen were selected for further studies. The five isolates pe16, pe68, pe74, pe88 and pe93 which showed multi drug resistance and more of protein production were selected for further studies (data presented elsewhere).

Cytotoxic effect of isolates:

Cultures were inoculated into nutrient broth and incubated for 24 hrs. After incubation cell extracts of the cultures were subjected to ammonium sulphate precipitation and dialysis using 1% sucrose solution [5]. Partially purified samples were subjected to micro-haemagglutination test to check cytotoxic effect of toxins. 1% suspension of sheep red blood cells in sterile physiological solution was added to each supernatant diluted by a factor of 2. This mixture was then incubated in 96 well micro-titre plates for 1 hour at 37ºC to determine α-haemolysis activity. The micro-titre plates were later incubated at 4ºC over night (hot-cold shock) to reveal β-haemolysis activity [6].

Genomic DNA extraction:

The genomic DNAs from five *E. coli* cultures were isolated using genomic DNA extraction Kit (Bhat Biotech). The pellet from 1.5 ml of overnight culture was resuspended in 500 µl of lysis buffer and incubated at 37ºC for 1 hour to lyse the cells. Genomic DNA was then extracted by Phenol/Chloroform. DNA from the aqueous phase was precipitated with isopropanol, washed with 70% Ethanol and air dried. The DNA pellet was dissolved in 50 µl of nuclease free water. 1 µl of the genomic DNA was used to analyze on 0.5% Agarose gel electrophoresis [7].

Polymerase Chain Reaction (PCR):

Amplification of the *Stx-1* and *Stx-2* gene was performed using following primer pairs [8].
Stx-1 Forward primer: 5'- ATGTCAGAGGGATAGATCCA -3'
Stx-1 Reverse Primer: 5'- TATAGCTACTGTCACCAGACAAT -3'
Stx-2 Forward primer: 5'- AGTTCTGCGTTTTGTCACTGT C -3'
Stx-2 Reverse Primer: 5'- CGGAAGCACATTGCTGATT -3'
PCR was performed as follows in a total volume of 50 µ in a 0.2 ml thin walled PCR tube.

Components	Quantity
Nuclease free water	37.0 µl
Genomic DNA (0.2µg/µl)	2.0 µl
Forward Primer (10µM)	2.0 µl
Reverse Primer (10µM)	2.0 µl

10X Reaction Buffer	5.0 µl
dNTP Mix (10mM)	1.5 µl
Taq DNA polymerase (5 U/µl)	0.5 µl
Total volume	50 µl

The amplification was carried out in a Master cycler® Thermocycler (Eppendorf, Germany) using initial denaturation of 95ºC for 2 minutes, followed by 40 cycles of denaturation at 94ºC for 45 seconds, annealing at 56ºC for 1 minute and extension at 72ºC for 1 minute. Final extension was carried out at 72ºC for 10 min. 10 µl of PCR product was analyzed on 1.5% Agarose gel electrophoresis.

RESULTS AND DISCUSSION

Out of 100 samples collected 74 samples showed the presence of *E. coli*. All the 74 isolates showed nucleated colonies with or without metallic sheen on EMB agar (**Fig. 1**). Among 74 isolates, isolates pe16, pe68, pe74, pe88 and pe93 showed comparatively more growth and more protein production.

When micro-titre plates with blood and partially purified proteins were incubated at 37ºC for 1 hr all five samples had changed their colour due to cytotoxicity. On 'hot-cold shock' treatment with overnight incubation at 4ºC all the five samples retained their colour and did not revert back to normal. This indicates that proteins from all the five isolates are capable of showing β-haemolysis blood cells (**Fig. 2**).

On genomic DNA isolation all the five isolates, pe16, pe68, pe74, pe88 and pe93, showed prominent bands on agarose gel (**Fig. 3**). On PCR analysis of the isolates there was no amplified product of 185 bp expected for *Stx-1* in any of the samples (**Fig. 4**). Only primer dimmers were observed in all samples. But there was an amplified product of 160 bp, corresponding to *Stx-2* in pe16 but not in other samples (**Fig. 5**). Band of ~520 bp was observed in samples 4 and 5, but that is not expected for *Stx-2*. This says that among the 5 *E. coli* isolates one isolate (pe16) is negative for *Stx-1* and positive for *Stx-2*, whereas the other four isolates (pe68, pe74, pe88 and pe93) are negative for both *Stx-1* and *Stx-2*.

The Biken test used by Honda *et al.* was 98% sensitive and 99% specific for the detection of *E. coli* isolates capable of producing heat-labile enterotoxin [2]. A toxin from an enterotopathogenic strain of *E. coli* H30 was purified and characterized by O'Brien and Laverk [3]. The *E. coli* H30 toxin and Shiga toxin showed similar bands on SDS-PAGE, identical isoelectric points, comparable biological activities, i.e., cytotoxicity, lethality for mice and enterotoxicity and the same relative heat stabilities. Nevertheless, the two toxins had apparently different molecular weights as determined by sucrose gradient analysis, by gel filtration and by cross-linking experiments with dimethyl suberimidate. Jeshveen *et al.* established a protocol for the detection of the pathogen *E. coli* O157:H7 and *E. coli* virulence genes (*eaeA, rfbE, hly, stx1* and *stx2*) in a multiplex PCR protocol using six specific primer pairs [4]. The target genes produced species-specific amplicons at 625 bp, 397 bp, 296 bp, 166 bp, 210 bp and 484 bp for *E. coli* O157:H7 (*fliCh7* gene) and virulence genes (*eaeA, rfbE, hly, stx1* and *stx2*) respectively.

Fig. 1: Growth of *E. coli* on Eosin Methylene Blue agar medium

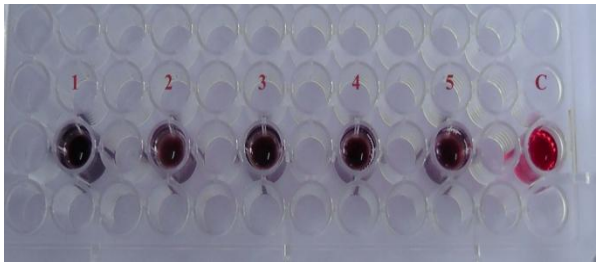

Fig. 2: Haemolytic effect of partially purified proteins from *E. coli* on blood cells in microtitre plate on 'hot-cold shock' treatment
Sample 1: pe16, Sample 2: pe68, Sample 3: pe74, Sample 4: pe88, Sample 5: pe93, Sample C: control

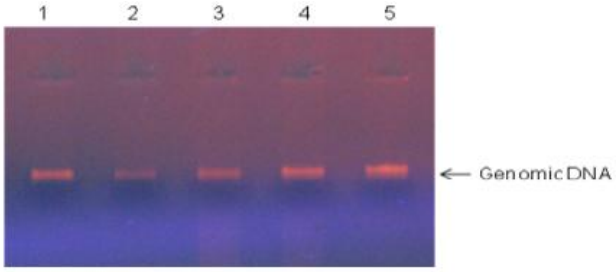

Fig. 3: Agarose gel electrophoresis of genomic DNA isolated from 5 different *E. coli* isolates. Lane 1: pe16, Lane 2: pe68, Lane 3: pe74, Lane 4: pe88, Lane 5: pe93

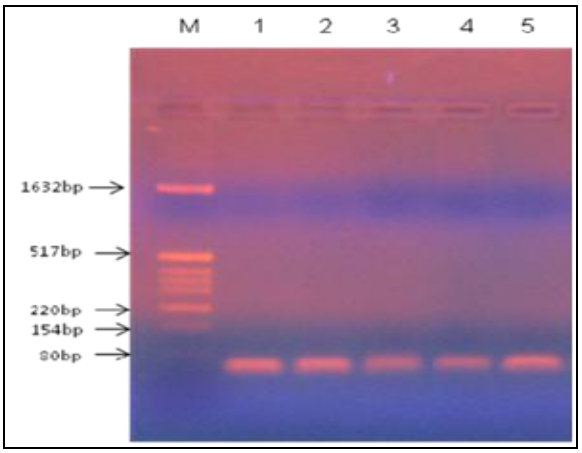

Fig. 4: Agarose gel electrophoresis of PCR products of *Stx-1*.
Lane M: DNA molecular weight marker; Lane 1: pe16, Lane 2: pe68, Lane 3: pe74, Lane 4: pe88, Lane 5: pe93

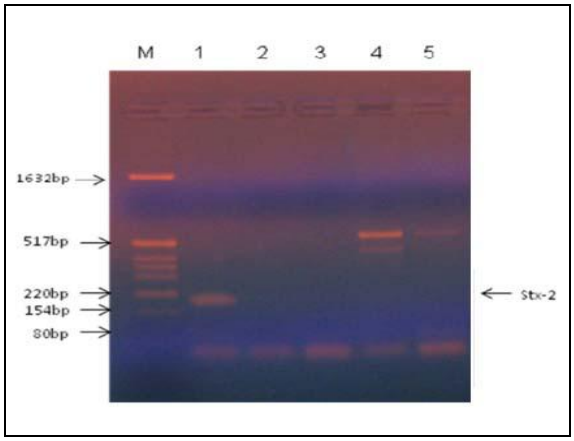

Fig. 5: Agarose gel electrophoresis of PCR products of *Stx-2*.
Lane M: DNA molecular weight marker; Lane 1: pe16, Lane 2: pe68, Lane 3: pe74, Lane 4: pe88, Lane 5: pe93

CONCLUSION

E. coli is prevalent in panipuri samples sold in Bangalore city. PCR analysis of the five isolates showed positive result for only stx2 toxin by only one isolate, whereas negative results were obtained for the other isolates. All the five isolates were negative for stx1 toxin. But cytotoxic effect of partially purified samples of all the five isolates showed β-haemolysis on blood agar. Though haemolysis may not be due to stx1 or stx2 toxins, it could be due to other toxins produced by the isolates. Productions of other types of toxins by the isolates need to be confirmed.

REFERENCES

1. Chapman P, Siddons CA, Cerdan Malo AT, Harkin MA. A one year study of *Escherichia coli* O157 in raw beef and lamb products. *Epidem. Infec.,* **2009**: 124; pp 207-213.
2. Honda T, Glass R, Akhtar Q, Kibriya AKMG. A simple assay to detect *Escherichia coli* producing heat-labile enterotoxin: Results of a field study of the Biken test in Bangladesh. *The Lancet,* **1981**: 318(8247); pp 609-610.
3. O'Brien AD, LaVeck GD. Purification and characterization of Shigella dysenteriae 1-like toxin produced by *Escherichia coli*. *Infec. Immun.*, **1983**: 40(2); pp 675-683.
4. Jeshveen SS, Chai LC, Pui CF, Son R. Optimization of multiplex PCR conditions for rapid detection of *Escherichia coli* O157:H7 virulence genes. *Internl. Food res. J.*, **2012**: 19(2); pp 461-466.
5. Rajan S, Christy RS. Experimental procedures in life sciences. 1st Edition. Anjanna Book House, Chennai: **2010;** pp. 153-156.
6. Albesa J. Bacterial haemolysins. *Adel. Microbiol. Enf. Infecc.,* **1998**: 7; pp 37-60.
7. Sambrook J, Fritsch EF, Maniates T. Molecular cloning: A laboratory manual, 2nd Edition, Cold Spring Laboratory Press, New York: **1989**; 2: pp 9.16-9.19.
8. Bélanger SD, Boissinot M, Ménard C, Picard FJ, Bergeron MG. Rapid Detection of Shiga Toxin-Producing Bacteria in Feces by Multiplex PCR with Molecular Beacons on the Smart Cycler. *J. Clin. Microbiol.,* **2002**: 40(4); pp1436-1440.

Source of support: Nil, Conflict of interest: None Declared