Detection of Escherichia coli O157:H7 prevalence in foods of animal origin by cultural methods and PCR technique

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Abstract

A total of 250 samples (50 each of beef, mutton and chicken and 50 samples each of beef swabs and mutton swabs) collected from various sources were subjected to PCR and cultural methods for the presence of Escherichia coli O157:H7. Primers for hlyA, stx1 & 2 genes were used for the detection of Escherichia coli O157:H7 and shiga toxins respectively. Out of 250 samples, 27 showed presence of Escherichia coli O157:H7 (5 beef, 6 beef swabs, 2 mutton, 12 mutton swabs and 2 chicken out of 50 samples each) by PCR where as only 11 samples (one beef, 2 beef swabs, 1 mutton, 6 mutton swabs and one chicken sample) were positive by cultural method. Of the 27 Escherichia coli O157:H7 positive samples by PCR, 12 showed stx1, 7 showed stx2 and 5 showed both stx1 and stx2. The sensitivity of PCR method for Escherichia coli O157:H7 was 1.7cfu. Enrichment with mTSB broth containing novobiocin gave good results compared to mEC broth with novobiocin by both PCR and cultural methods.

Keywords: Escherichia coli O157:H7, PCR, Laboratory Technique, Prevalence, Cultural method.

Introduction

Escherichia coli O157:H7 is an important emerging human pathogen causing Haemmorhagic Colitis, Haemolytic Uraemic Syndrome and Thrombotic Thrombocytopaenic Purpura (Nataro and Kaper, 1998; Zhao et. al., 1998). Escherichia coli O157:H7 serotypes are identified as enterohaemorrhagic E. coli (Oksuz et. al., 2004). In 1982, an investigation by the Centres for Disease Control and Prevention (CDC, United States) of two severe bloody diarrhea outbreaks associated with a fast food restaurant led to identification of unique E.coli strain, O157:H7 (Wells et. al., 1983). The infections by Escherichia coli O157:H7 have been reported of increasing frequency from all parts of the world in the form of food poisoning outbreaks (Jo et. al., 2004). Because of the severity of these illnesses and the apparent low infective dose (< 10 cells, Bach et. al., 2002), Escherichia coli O157:H7 is considered one of the most serious of known foodborne pathogens (Blanco et. al., 2003).

Escherichia coli O157:H7 strain produces shiga toxin 1 (stx-1) and shiga toxin 2 (stx-2) which are also referred as verotoxins. Escherichia coli O157:H7 strains carrying stx genes along with enterohaemolysin (hlyA) and intimin (eae) genes are potentially dangerous to human health (Manna et. al., 2006).

Cattle are the biggest reservoir of E. coli O157:H7 and beef and milk contaminated with cattle faeces are the most common sources of human infection (Armstrong et. al., 1996). E. coli O157:H7 is a foodborne pathogen that has been associated with meat products (Sivapalasingam et. al., 2004) particularly associated with the consumption of undercooked ground beef (Doyle, 1991). 52% of *E. coli* O157:H7 outbreaks have been associated with bovine products (WHO, 1997).

The conventional isolation procedure includes growth in enrichment broths like mEC broth (Okrend et. al., 1990) or mTSB broth (Doyle and Schoeni, 1987) both supplemented with novobiocin at 370C for 18-24 hours to increase the ratio of Escherichia coli O157:H7 to competitor organisms. The broth cultures are then plated onto Sorbitol MacConkey agar supplemented with cefixime-tellurite (CT). Escherichia coli O157:H7 is unable to ferment sorbitol resulting in colourless colonies. So, non-sorbitol fermenting (NSF) colourless colonies are potentially considered as *E. coli* O157:H7 (March and Ratman, 1986; Mc Donough et. al., 2000). CT supplement increases the sensitivity of this medium and yields best results for selective

cultivation of Escherichia coli O157:H7 over other E. coli and non-sorbitol fermenters such as Aeromonas, Pleisomonas, Morganella and Providencia (Chapman et. al., 1991; Sanderson et. al., 1995; Heuvelink et. al., 1997; De Boer and Heuvelink, 2000).

This work has been undertaken to detect the presence of Escherichia coli O157:H7 in some of the foods of animal origin using both PCR and cultural methods and to investigate the presence of shiga toxins 1 and 2 (stx1 and stx2).

Materials and methods

A total of 150 different meat samples (50 samples each of beef, mutton and chicken) and 100 meat surface swabs (50 each of beef swabs and mutton swabs) were collected from freshly dressed and washed animal carcasses at slaughter houses and markets in Hyderabad. Meat samples (10gm each) and swabs were enriched in 90 ml of modified Escherichia Coli (mEC) broth and modified Tryptic Soy broth (mTSB) both supplemented with novobiocin at 370C for 18 hours. The broth cultures were spread plated onto MacConkey Sorbitol agar containing Cefixime-Tellurite supplement at 420C for 24 hours for isolation of Escherichia coli O157:H7. The sorbitol negative colourless colonies were taken for further confirmation by biochemical tests like IMViC (Indole, Methyl Red, Voges Proskeur and Citrate utilization) tests, nitrate, lysine decarboxylase, ONPG (Ortho-nitrophenyl galactosidase) and sugar fermentation tests (ferments sucrose, maltose, lactose, mannitol and mannose but does not ferment sorbitol and cellobiose).

All the enriched samples were subjected to PCR analysis for the presence of Escherichia coli O157:H7 using primers specific to haemolysin gene (hlyA). The samples positive for Escherichia coli O157:H7 by PCR method were further examined for the presence of shiga toxins (stx1 and stx2) using specific primers (Table.1).

An Escherichia coli O157:H7 strain, obtained from National Institute of Enteric diseases, Kolkata was used as known positive strain in PCR analysis. 1.5 ml of enriched broths were taken into eppendorf tubes and bacteria were pelleted by centrifugation at 6000rpm for 5 min. To the pellet 50µl of molecular grade water was added and incubated at 650C for 15 min. and snap chilled to release DNA. Then centrifuged at 13000rpm for 5 min. and the supernatants were used as DNA templates for PCR analysis.

Bacterial DNA amplification was done in 20µl reaction mixture containing 2µl of 10X Taq DNA polymerase buffer (containing 100mM Tris with PH 9.0, 500mM KCl, 15mM MgCl2 and 1% Triton X-100), 2µl of 10mM of dNTP mix, 0.9U of Taq DNA polymerase (Genei), 2µl each of 4 pmoles/µl of forward and reverse primers and 5µl of crude bacterial cell lysate. Make this mixture to 20µl using molecular grade water. Amplification was done in thermal cycler following standardized conditions. (Table.2).

The amplified DNA fragments were resolved by agarose gel electrophoresis, stained with ethidium bromide (0.5µg/ml) and visualized with an UV transilluminator (Fig.1).

Spiking studies: To know the sensitivity of PCR method for Escherichia coli O157:H7, homogenized beef was inoculated at the rate of 170cfu, 17cfu, 1.7cfu and 0.17cfu/10gm of beef and transferred to two different enrichment media i.e. modified EC broth and modified Tryptic Soy broth. The PCR and cultural testing were carried after 10hr and 18hr of incubation.

Results and Discussion

The detection level of Escherichia coli O157:H7 was 17cfu and 1.7cfu for mEC broth after 10hr and 18hr incubation respectively, where as it was 1.7cfu for mTSB after 10hr as well as 18hr incubation using PCR. But it was 170cfu and 17cfu for mEC and 17cfu and 1.7cfu for mTSB after 10hr and 18hr incubation respectively by cultural method. Almost similar results were reported by Arthur et. al. (2005) using mTSB with minimum detection level of 1.7cfu by both PCR and cultural method after 18hr incubation.

The growth of Escherichia coli O157:H7 in samples as well as spiking studies was good in mTSB compared to mEC. Restaino et. al. (1996) reported that mTSB took less time for doubling of Escherichia coli O157:H7 (28 min) than mEC (58 min).

The results for the presence of Escherichia coli

Table-1. Oligonucleotide primers used in the study.

Primer	Target gene	Primer sequence (5`-3`)	Fragment size (bp)	Reference
O157-3	hlyA	GTA GGG AAG CGA ACA GAG	361	Wang et. al., 1997
O157-4	hlyA	AAG CTC CGT GTG CCT GAA	361	Wang et. al., 1997
Stx1-F	stx1	ACA CTG GAT GAT CTC AGT GG	614	Manna et. al., 2006
Stx1-R	stx1	CTG AAT CCC CCT CCA TTA TG	614	Manna et. al., 2006
Stx2-F	stx2	CCA TGA CAA CGG ACA GCA GTT	779	Manna et. al., 2006
Stx2-R	stx2	CCT GTC AAC TGA GCA CTT TG	779	Manna et. al., 2006

Table-2. Cycling conditions used for three sets of primers

No.	Step	hly A (Escherichia coli O157:H7)	stx1 and stx2 (STEC)
1.	Initial denaturation	940C/5min	940C/5min
2.	Final denaturation	940C/1min	940C/1min
3.	Annealing	520C/1min	600C/1min
4.	Initial extension	740C/2min	720C/2min
5.	Final extension	740C/10min	720C/10min

O157:H7 in different meat samples are presented in Table.3. Escherichia coli O157:H7 was isolated from 10% (5 out of 50) of beef samples by PCR and 2% (1 out of 50) by cultural method. Almost similar results of 14.7% and 17% were reported in beef by Mora et. al. (2007) and Wilshaw et. al. (1993) respectively. Higher incidence of 31% in beef was reported by Doyle and Schoeni (1987) and 35.8% by Elder et. al. (2000). Low levels of 0-3.7% was reported in ground beef by Desmarchelier and Grau (1997). The swab samples taken from the surface of beef carcasses yielded 12% (6 out of 50) and 4% (2 out of 50) by PCR and cultural methods respectively. Low levels of 3.7% was reported in beef swab samples by Manna et. al. (2006).

Escherichia coli O157:H7 was isolated from 4% (2 out of 50) of mutton samples by PCR and 2% (1 out of 50) by cultural method where as in mutton swabs it was 24% (12 out of 50) and 12% (6 out of 50) by PCR and cultural methods respectively. Doyle and Schoeni (1987) reported 2% incidence in mutton. Chapman (2000) reported higher incidence of Escherichia coli O157:H7 in lamb products than beef products. Escherichia coli O157:H7 were isolated form 4% (2 out of 50) of chicken samples by PCR and from 2% (1 out of 50) of samples by cultural methods. Almost similar results were reported by Doyle and Schoeni (1987) and Desmarchelier and Grau (1997). Compared to meat samples, meat swabs yielded more positive results by both PCR and cultural methods. This may be due to the probable faecal contamination during slaughter.

For a total of 250 samples (meat and meat swabs), cultural method yielded 4.4% positive results where as for PCR it was 10.8%. So, PCR method is more accurate than cultural method for the isolation of Escherichia coli O157:H7. Similar opinion was given by Weagent et. al, (1995).

Of the 27 samples positive for Escherichia coli O157:H7 by PCR method, 12 (44.4%) showed presence of stx1, 7 (26%) showed stx2 and 5 (18.5%) showed both stx1 and stx2. Incidence of stx1 was high compared to stx2 and it is in co-ordination with the reports of Cerqueira et. al. (1997) and Sidjabat-Tambunan et. al. (1998) which showed higher production of stx1 during summer.

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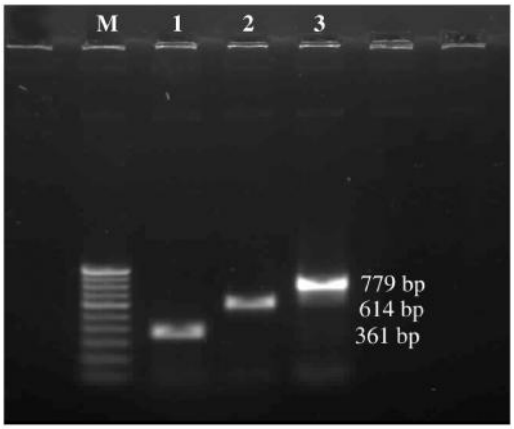

Fig. 1: Results of the PCR assay
Lane M: 100-bp DNA marker
Lane 1: amplifying 361-bp segment of hly A
Lane 2: amplifying 614-bp segment of stx 1
Lane 3: amplifying 779-bp segment of stx 2

Table-3. Occurence of Escherichia coli O157:H7 in different meat samples

S.No.	Sample	No. of samples	Results for E. coli O157:H7		stx1	stx2	Both stx1 & stx2
			Cultural	PCR			
1.	Beef samples	50	1	5	2	1	1
2.	Beef swabs	50	2	6	2	2	1
3.	Mutton samples	50	1	2	1	1	1
4.	Mutton swabs	50	6	12	6	3	2
5.	Chicken samples	50	1	2	1	0	0

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