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Short Paper

Isolation of enterotoxigenic and enteroaggregative straines of *Escherichia coli* from chicken carcasses by PCR

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Summary

The aim of this study was to determine the frequency of enterotoxigenic and enteroaggregative strains of *Escherichia coli* in chicken carcasses by polymerase chain reaction (PCR). In this study 63 strains of *E. coli* were isolated from 110 samples of chicken carcasses during processing after chilling in the poultry slaughter house of Shahrekord. Polymerase chain reaction assays were used to detect the presence of the genes encoding heat-stable enterotoxin a (STa), heat-stable enterotoxin b (STb), heat labile toxin (*LT*) and Enteroaggregative heat-stable toxin 1 (EAST1). Sixty three out of 110 (57.27%) carcasses were contaminated with *E. coli*. Six out of 63 (9.52%) harbored the gene for *LT*, 1 (1.58%) *STb*, 21 (33.3%) *EAST1* and 8 (12.69%) contain both *LT* and *EAST1* genes. None of the strains contain the *STa* gene. The results indicated that contamination of the chicken carcasses with *E. coli* in such a level could be a potential hazard for consumers.

Key words: Chicken carcasses, *Escherichia coli*, ETEC, EAggEC, PCR

Introduction

Escherichia coli is a Gram-negative bacterium belonging to the family of *Enterobacteriaceae*.

Escherichia coli is part of the normal intestinal flora of humans and animals. Pathogenic strains of *E. coli* bacteria can potentially cause intestinal and extraintestinal infections in mammalian and avian hosts (Cullor, 1996). Up to now, several classes of enterovirulent *E. coli*, namely enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diarrhoea-associated haemolytic *E. coli* and cytolethal distending toxin (CLDT)-producing *E. coli* have been recognized (Nataro and Kaper, 1998). Ingestion of contaminated water or food results in ETEC infection, producing watery diarrhea, nausea, abdominal cramps and low-grade fever (Raj, 1993; Nweze, 2009). Two enterotoxins, heat labile toxin (*LT*) and heat-stable (*ST*), play a distinct role in the pathogenesis of Enterotoxigenic strains (Pohl *et al*., 1993). *LT* is inactivated at 60°C for 15 min. The genes encoding *LT* (*elt or etx*) reside on a plasmid which may also harbor genes encoding *ST* and/or colonization factor antigen (CFA) (Gill *et al*., 1981). There are two classes of heat-stable toxins, *STa* (*STI*) and *STb* (*STII*), which differ structurally and functionally.

They are small monomeric toxins resistant to heat treatment at 100°C for 15 min. The genes encoding both these toxins are present on plasmids (Nataro and Kaper, 1998; Gyles and Fairbrother, 2004).

EAggEC strains are associated with acute or persistent diarrhea among children in tropical and nontropical temperate regions and have been implicated in food-borne outbreaks, nosocomial infections and travelers' diarrhea via producing enteroaggregative heat-stable toxin 1 (*EAST1*), this is a 4.1 kDa peptide sharing 50% homology with *STa* (Savarino *et al.*, 1996; Vila *et al.*, 2000; Nishikawa *et al.*, 2002). It has been proposed that the mechanism of action of *EAST1* is similar to *STa* in increasing cyclic Goanosine monophosphate (cGMP), however, the exact role of *EAST1* in the development of diarrhea is still unclear (Nataro and Kaper, 1998; Gyles and Fairbrother, 2004).

The objectives of this study were to investigate the ETEC and EAggEC and isolate their characterization from chicken carcasses slaughtered in the Shahrekord poultry slaughter house by PCR method.

Materials and Methods

Swab samples were obtained from 110 carcasses randomly during processing after chilling in the poultry slaughter house of Shahrekord. The samples (in TSB) were transported to the laboratory inside an ice box (4°C) within 2 h, and cultured on MacConkey agar (Merck, Germany). All plates were incubated at 37°C for 24 h. Colonies showing *E. coli* characteristics were submitted to Gram staining and identified by standard biochemical tests i.e. oxidase, indole, Simon's citrate, urease and hydrogen sulfide (Varnam, 1991).

Sixty three strains of *E. coli* were isolated from chicken carcasses. PCR assays were used to detect the presence of the genes encoding *STa*, *STb*, *LT* and *EAST1* toxins by using specific primers (Metabiun Laboratoy, Germany), (Table 1). The culture of each isolate was prepared by inoculating in Tryptose soy broth (Merck) and incubated at 37°C for 24 h, an aliquot was diluted in 450 µl of distilled water and boiled for 10 min. Then it was centrifuged (Seward) at 3000 g for 2 min and supernatant was taken as DNA template.

PCR was carried out in a final reaction volume of 23 µl using 0.2 ml thin wall PCR tube. A master mix (25 ml PCR buffer 10X, 10 ml $MgCl_2$, 7.5 ml of dNTPs and 1.5 ml of *Taq* DNA polymerase) for a minimum of 10 samples was prepared and dispersed into PCR tubes and 2 µl sample of DNA was added in each tube to make the final volume of 23 µl. PCR tubes containing the mixture were tapped gently and quickly spin at 10,000 rpm for a few seconds. The PCR tubes with all the components were transferred to a thermal cycler (Biorad, USA) and the thermal cycle was done as mentioned in Table 2.

To confirm the targeted PCR

Table 1: Characterization of primers using in this study (Blanco *et al.*, 2004)

Primers	Sequences (5'- 3')	Size (bp)	Aneal. T (°C)	Positive control
LT-F	TTA CGG CGT TAC TAT CCT CTC TA	275	60	P97-2554B
LT-R	GGT CTC GGT CAG ATA TGT GAT TC	275	60	O149:K91
STa-F	TCC CCT CTT TTA GTC AGT CAA CTG	163	60	P97-2554B
STa-R	GCA CAG GCA GGA TTA CAA CAA AGT	163	60	O146:K91
STb-F	GCA ATA AGG TTG AGG TGA T	368	60	P97-2554B
STb-R	GCC TGC AGT GAG AAA TGG AC	368	60	0149:K91
EAST1-L	TCG GAT GCC ATC AAC ACA GT	125	55	P97-2554B
EAST1-R	GTC GCG AGT GAC GGC TTT GTA G	125	55	0149:K91

Table 2: Thermal cycle using for amplification of *LT*, *STa*, *STb* and *EAST1* genes (Blanco *et al.*, 2004)

Genes	Cycle	Denaturation	Annealing	Extension	Number of Cycle	Final Extension
LT & STa & STb	Temperature	94	60	72	30	72
	Time (min)	4	1	1		2
EAST1	Temperature	94	55	72	30	72
	Time (min)	4	1	1		2

Table 3: Virulence genes of *E. coli* isolated from chicken carcasses

Number of samples	*E. coli*	*EAST1*	*STb*	*LT*	*LT & EAST1*	*LT & STb*	*STa*
110	63 (57.27%)	21 (33.3%)	1 (1.58%)	6 (9.52%)	8 (12.69%)	1 (1.58%)	0 (0.0%)

amplification, 10 µl of PCR product from each tube was mixed with 1 µl of 6 X gel loading buffer from each tube and loaded into the well of 1% agarose gel along with 100 bp DNA Ladder (Gene Ruler-Fermentas), stained with ethidium bromide (Merck) and electrophoresed at constant 98 V for 80 min in 0.5 X TBE buffer (Cinnagen). The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (UV Tec, UK).

Results

Sixty three isolated *E. coli* strains from chicken carcasses were submitted to PCR for detection of 4 virulence genes. The total number of potentially virulent strains identified were 37 from 63 investigated strains (58.73%), (Table 3). Regarding the results of PCR tests, 6 out of 63 (9.52%) of the isolates harbored the gene for *LT*, 1 (1.58%) *STb*, 21 (33.3%) *EAST1* and 8 (12.69%) contained both *LT* and *EAST1* genes. None of the strains contained the *STa* gene (Table 3) (Fig. 1).

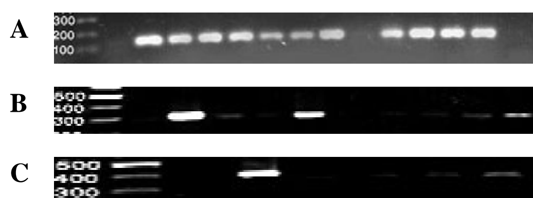

Fig. 1: PCR products of *EAST1*, 125 bp (A), *LT*, 275 bp (B) and *STb*, 368 bp (C) genes, marker 100 bp

Discussion

In the present study, we have established the virulence genes of *E. coli* isolated from chicken carcasses. This study revealed that virulence genes were commonly presented in *E. coli* strains isolated from chicken carcasses. It is important to emphasize the need to routinely screen for virulence markers in *E. coli* strains isolated from food, regardless of whether they have been serogrouped as EPEC or not.

Despite the role of food as a vehicle of ETEC in outbreaks and sporadic diarrheal cases worldwide, in the present study this category of enterotoxigenic *E. coli* was determined in 25.2% of potentially virulent isolates. Moreover, EAEC isolates that produce a low-molecular-weight, partially heat-stable, plasmid encoded enterotoxin named enteroaggregative heat-stable enterotoxin 1 (EAST1) (Nataro and Kaper, 1998), were detected in 33.3% of potentially virulent strains. Previous reports have confirmed that food, as a vector for transmission of EAEC, has little significance (Cerqueira *et al*., 1999).

The result reported here is a survey of the incidence of potentially virulent *E. coli* strains in chicken carcasses. Conditions experienced during their production present a risk of microbial contamination. Conventional microbiological diagnostics in food control includes only the determination of *E. coli* numbers per gram without any other characterization of isolated strains. According to our study, regarding the incidence of potentially virulent strains of *E. coli*, it was important whose outcome would be forwarded to the authorities to take the necessary measures to protect consumer health like destruction or recall of a contaminated lot of food. Poor hygienic practices may contribute to increasing the prevalence of pathogens in foods, thereby increasing the risk of food borne disease for consumers (Cohen *et al*., 2006). Results obtained in this study provide evidence which may be used by the government to adopt regulation enforcing the application of the hazard analysis critical control point (HACCP) system as a means to identify and control the hazards in poultry slaughter houses.

Most of the previous studies focused on verotoxigenic (VTEC) strains of *E. coli* and revealed the potentially hazard exposure of the human population to these strains due to the prevalence of the strains in chicken carcasses (Heuvelink *et al*., 1999; Tutenel *et al*., 2003; Akkaya *et al*., 2006; Ghanbarpour

et al., 2010).

Additional research about the virulence markers present in *E. coli* strains from humans in Iran is certainly needed to better understand the relative importance of the molecular features of potentially virulent *E. coli* strains revealed by our results in causing disease based on strain characteristics from *E. coli* isolates from human patients.

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