

PCR detection and serotyping of enterotoxigenic and shigatoxigenic *Escherichia coli* isolates obtained from chicken meat in Mumbai, India

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Abstract

Aim: Present study was undertaken to find out the frequency of few virulent genes and prevalence of related strains of *Escherichia coli* isolated from chicken meat obtained from chicken retail shops by Polymerase Chain Reaction (PCR).

Materials and Methods: 66 samples of freshly slaughtered chicken meat were collected from 22 identified retail shops located at Mumbai city, randomly. Processed meat samples were cultured in EMB agar and presumptive colonies were confirmed by various biochemical tests. PCR method was accustomed for identification of the genes coding for heat-stable enterotoxin a (STa), heat labile enterotoxin (LT), shiga-like toxins 1 and 2 (SLT1 and SLT2). *E. coli* isolates were sent to National Salmonella and Escherichia Centre, CRI, Kasauli, HP, India for serotyping.

Results: 11 (16.67%) *E. coli* strains were isolated from 66 chicken meat samples. 3 (27.27%) out of 11 harbored the gene for SLT2, and 2 (18.18%) for STa. None of the strain contains SLT1 and LT genes. Serotypes detected were rough, O2, O20, O22, O102 each for one isolate and 6 isolates were untypable (UT).

Conclusion: The results concluded that chicken meat samples analysed harbored genes for shiga like toxins and enterotoxins and different serotypes of *E. coli*. These findings indicating that regular monitoring of chicken meat is essential for this pathogen to prevent potential public health problems.

Keywords: chicken meat, enterotoxin, *Escherichia coli*, polymerase chain reaction, virulence gene

Introduction

Escherichia coli strains are usually recovered from intestinal tracts of human, poultry, animal and commonly present in soil, water and foods due to faecal contamination or contamination during food animal slaughter. They are commonly present in nature and has major role in maintaining equilibrium of intestinal physiology of poultry [1]. Some *E. coli* strain causes several human diseases like haemolytic uremic syndrome (HUS), haemorrhagic colitis (HC) etc. and various isolates established as major pathogen for poultry colibacillosis [2, 3]. It is recognized as major pathogen for public health problems in developing countries and represents leading etiological agent of diarrhea [4].

Several classes of *E. coli*, specifically enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC) or enterohaemorrhagic *E. coli* (EHEC) or verotoxin producing *E. coli* (VTEC), enteroaggregative *E. coli* (EAggEC) have been recognized [4]. Enterotoxigenic *E. coli* infection owing to ingestion of contaminated food or water produces abdominal cramps, low-grade

fever, watery diarrhea and nausea [5]. Heat-stable (ST) and heat labile (LT), these two enterotoxins, produced by this category plays a distinct role in the pathogenesis. Two classes of heat-stable toxins, STa (STI) and STb (STII), differ structurally and functionally. The genes encoding both these toxins are present on plasmids [6]. Shiga-like toxin-producing *E. coli* strains, responsible for HUS, HC, diarrhea and renal failure in children, are zoonotic water and food borne pathogens [7]. Previous reports revealed that most outbreaks of HUS and HC are caused by serotype *E. coli* O157:H7 [8]. STEC produces two types of shiga-toxins, SLT1 and SLT2. These toxins have similar biological functions, molecular structure and encode different set of genes in which most of genes are virulence associated so that STEC contaminated food could be at risk for public health. There are several techniques for detection of toxins in which PCR widely taken for targeting SLT-specific genes in many studies [9, 10]. Therefore, this study was designed taken to find out the frequency of ETEC and STEC virulent genes isolated from chicken meat slaughtered in the retail chicken shops by PCR method.

Materials and Methods

Sample collection: In present study, 22 identified retail shops {licensed by Brihanmumbai Municipal Corporation (BMC)} were selected from Mumbai city

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Table-1. Oligonucleotide primer sets used in PCR

Gene	Sequence (5'-3')	Annealing temp. (°C)	Product size (bp)	References
SLT1	F-GAAGAGTCCGTGGGATTACG (20) R-AGCGATGCAGCTATTAATAA (20)	55	130	12
SLT2	F-TTAACCACACCCACGGCAGT (20) R-GCTCTGGATGCATCTCTGGT (20)	55	346	12
STa	F-GGGTTGGCAATTTTATTCTGTA (24) R-ATTACAACAAAGTTCACAGCAGTA (24)	59	183	13
LT	F-TAGAGACCGGTATTACAGAAATCTGA (26) R-TCATCCCGAATTCTGTTATATATGTC (26)	59	282	13

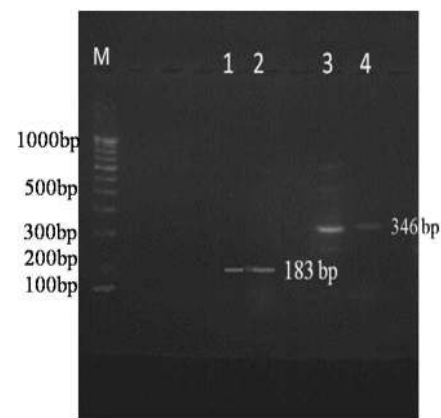


Figure-1. SLT2 gene and STa gene of *E. coli*
Lane M: 100 bp ladder
Lane 1 & 2: STa gene, 183 bp
Lane 3 & 4: SLT2 gene, 346 bp

and 66 samples of chicken meat were collected randomly (Random Sampling Method). From these samples, muscle pieces were separated into sterile bags by using sterile forceps and scissors and transported by maintaining cold.

Identification of *E. coli*: The surfaces of chicken muscles were incised with scissor and 10 gm muscle was mixed with 90 ml of NSS in sterile bag. Thereafter, serially diluted the sample from 10^{-1} to 10^{-5} then cultured on Eosin Methylene Blue (EMB) agar (HiMedia, India) and incubated for 18 to 24 h at 37 °C. Colonies with green metallic sheen were considered *E. coli* and biochemically tested for indole production, glucose degradation, tryptophan degradation, citrate utilization, urease production and for motility and growth on triple sugar iron agar (TSI). These isolates of *E. coli* were stored in Nutrient agar (HiMedia, India) slants till use.

DNA extraction: DNA of *E. coli* was isolated by Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Wilson [11] with slight modifications. Five ml LB broth (HiMedia, India) culture of *E. coli* were incubated at 37°C overnight. Three ml culture was taken in microcentrifuge tube and pelleted by centrifugation at 10,000 rpm for 5 minutes. Pellet was resuspended in 567 µl of TE buffer, added 0.5% SDS (SRL, India) and 100 µg/ml proteinase K (SRL, India), mixed thoroughly and incubated for 1 hour at 37°C. Added 100 µl of 5 M NaCl (SRL, India) to cell lysate and mixed thoroughly. Thereafter, 80µl of CTAB/NaCl (SRL, India) solution was added and incubated at 65°C for 10 minutes. Equal volume (0.7 to 0.8ml) of Phenol/Chloroform/Isoamyl-alcohol (25:24:1) was added and centrifuged for 5 minutes at 13000 rpm. Thereafter, the upper aqueous, viscous supernatant was transferred to fresh tubes then 0.5 volume of 7.5 M ammonium acetate (SRL, India) and double amount of chilled absolute ethanol were added. The tubes were kept at -20°C for overnight, obtained pellet by centrifugation for 20 minutes at 4°C at 13,000 rpm and washed with 70% ethanol (SDFCL, India). The pellet

was dried by keeping tubes open and resuspended in 50 µl of elution buffer. Finally, DNA integrity was assessed by running in 1.7% agarose (HiMedia, India) gel. Extracted DNA was stored at -20°C till further use.

Primers: The presence of virulence associated genes i.e. shiga-like toxin (SLT1 and SLT2), heat stable toxin (STa) and heat labile toxin (LT) of *E. coli* were determined by PCR. Primers used for associated genes are depicted in Table-1.

Detection of virulent genes by PCR: The PCR reaction for amplification of SLT1, SLT2, STa and LT genes was done in standard 25 µl reaction in 0.2 ml PCR tube (Axygen, USA), separately. 2 µl of DNA was used as template. 20 picomolar solution of each primer (Sigma, India), 1X Taq amplification buffer, (SRL, India), 1mM MgCl₂, 200 µM each nucleotides (SRL, India) and 1.25 units of Taq DNA polymerase (SRL, India) were used for each reaction. The mixture containing PCR tubes were quickly spun at 10000 rpm for few seconds and placed in gradient thermal cycler (Eppendorf, Germany). The cycling conditions for amplification included 94°C for 3 minute (initial denaturation), 30 cycles of 94°C for 1 minute (denaturation), annealing for 1 minute (annealing temperature shown in Table 1) and 72°C for 1 minute (polymerization) followed by 72°C for 10 minute (final extension). The positive amplification was visualized by electrophoresis of the product in 1.7% agarose (HiMedia, India) gel stained with ethidium bromide in a submarine horizontal electrophoresis apparatus (Bangalore Genei).

Serotyping of *E. coli*: *E. coli* isolates obtained from chicken meat were sent to Central Research Institute, Kasauli, HP, for serotypes differentiation.

Results

Out of 66 chicken meat samples analysed, 11 (16.66%) samples showed presence of *E. coli* isolates (Incidence 16.66%). Regarding the results of PCR, shiga like toxin-2 was found in 3 (27.27%) and heat stable toxin in 2 (18.18%) of the 11 positive *E. coli*

isolates (Figure 1). In other words, frequency of shiga like toxin and heat stable toxin gene was 27.27% and 18.18% respectively. None of the heat labile toxin gene associated *E. coli* isolates was identified in this study. The total identified virulent strains were 5 among 11 investigated strains (45.45%). As a result, overall incidence of virulent strains of *E. coli* was 7.57% (ETEC, 3.03% and STEC, 4.54%) (5 virulent strains out of 11 *E. coli* strains isolated from 66 samples). In addition, all *E. coli* strains were serotyped in which 5 isolates were confirmed as rough, O2, O20, O22 and O102 while rest of the 6 isolates were untypable (UT).

Discussion

The prevalence of ETEC and STEC strains and frequency of associated virulent genes were explored among *E. coli* isolates obtained from chicken meat (collected from chicken retail shops) in Mumbai city, India. Virulent genes of *E. coli* strains were commonly found in chicken meat, which necessitate routinely monitoring of *E. coli* strains isolated from food. In the present study, frequency of ETEC associated genes was found 18.18% of total isolates. Similar study carried out in Iran, there frequency of ETEC strains associated genes were 25.2% reported, which is not in agreement with the our findings wherein frequency was lower [14]. This type of variance may be because of small sample size under the present investigation. Moreover, 3.03% incidence was observed for enterotoxigenic *E. coli* obtained from 66 samples of chicken meat. Similar to this observation, previous reports from India have varied findings for prevalence of ETEC ranging from 0.92% to 12% [15-17]. In India information regarding the STEC is available on cattle, sheep, fish, human feces but information related to STEC prevalence in chicken meat is rare. In the present study STEC associated genes frequency was found 27.27% of total isolates. In this investigation, very low proportion of STEC strains were isolated (Incidence 4.54%). These data is consistent with the low prevalence of STEC infection in India [17, 18]. Previous reports have confirmed that food transmission of STEC has little significance, estimated prevalence ranges from 0 to 11.1% [19, 20]. Along with, most studies focused on STEC strains of *E. coli* revealed that chicken meat is a major source for exposure of these strains to human population [21-23]. Here prevalence of ETEC and STEC strains and frequency of associated virulent genes of *E. coli* strains in chicken meat was reported. In traditional microbiology usually *E. coli* determination carried out as numbers/gram without characterizing the strains isolated. But in this study incidence of virulent strains of *E. coli* showed that there is need for necessary measures to protect consumer health by forwarding these necessary information to higher authorities and thus to facilitate necessary action required to protect public health by preventing poor hygienic practices that increases the prevalence of food pathogens [24].

Conclusion

The results concluded that chicken meat samples analyzed were harbored virulent genes for shiga like toxins and enterotoxins. It pointed out that chicken meat contaminated with *E. coli* strains in such level could produce potential hazard for consumers. Next to that, further exploration of other virulent strains like enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAaggEC) are needed to know the particular situation of *E. coli* contaminated chicken meat at local market.

Authors' contributions

RJZ, DMC, PRS executed the study design and analyzed the data. RJZ supervised the work and edited the paper. DMC collected samples and interpreted the results. PRS and AKR performed the experiments. AKR drafted and revised the manuscript. VMV assisted in the designing and performance of PCR. All authors discussed implications, results and finalized the manuscript. All authors read and approved the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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