CHARACTERIZATION OF *E. COLI* ISOLATES OF BUFFALO MEAT ORIGIN FOR *papC* VIRULENCE GENE

K.K. Kunal, Daljeet Chhabra and S.B. Barbuddhe
Department of Veterinary Microbiology
College of Veterinary Sc. and A.H., Mhow (M.P.)
@Corresponding author : daljeetchhabra@rediffmail.com
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ABSTRACT

Twenty nine *E. coli* isolates belonging to 12 serotypes and rough strains recovered from buffalo meat were characterized for *papC* gene. The PCR assay was standardized and the primer set for this gene allowed amplification of 501bp. Amplified product of the gene viz. *papC* was revealed in 23 (79.3%) *E. coli* isolates belonging to 12 serogroups O12, O24, O35, O60, O69, O88, O114, O139, O159, O168, O169, O172 and rough strain.

KEYWORDS : *E. coli, papC* gene, buffalo meat

INTRODUCTION

Amongst various food pathogens pathogenic strains of *E. coli* occupies a key position causing intestinal disorders and septicemia in animals and human beings. *E. coli* is a common contaminant found in meat acquired either from slaughtered animal, slaughter house or during various stages of meat processing (Yadav *et al.,* 2006).

Various virulence factors like enterotoxins, haemolysins, cytotoxins, fimbrial antigens and invasiveness have been characterized. These multiple virulent factors facilitate the ability to adhere, proliferate and colonize the small intestine and invasion of host, as well as the capacity to produce toxins, avoidance or disruption of host defense mechanisms, injury to host tissues and/or stimulation of a noxious host inflammatory response contribute to the pathogenicity of *E. coli* organisms (Dezfulian *et al.,* 2003).

Meat and their products often have a very complex microflora, which makes isolation procedure difficult. Therefore, the molecular techniques like PCR is adopted which allow screening and specific detection of organisms from clinical samples or contaminated food materials in short time and characterization of their virulent associated genes. The present research work was taken up with a view to detect virulent gene which might be associated with virulence of *E. coli,* isolated from buffalo meat sample.

MATERIALS AND METHODS:

The study was conducted on twenty nine isolates of *E. coli*, recovered from buffalo meat which was maintained at the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Mhow.

In the present study, readymade kit was used for template DNA preparation (Chromous Biotech Pvt. Ltd., Banglore). Template DNA obtained by this kit yielded good results in PCR. This kit proved to be a very simple and rapid technique for template DNA preparation.

The PCR was standardized for the detection of virulent gene (*papC*) of *E. coli* which encode for p-fimbriae, using suitable primers (Table 1) as described by Ewers *et al.* (2003) with suitable modifications.

Table 1: Details of primers used for PCR reaction

Primers	Sequences (5'-3')	Target gene	Size of amplified product(bp)	Reference
papC(F)	5'-TGATATCACGCAGTCAGTAGC-3'			Ewers *et al*.
papC(R)	5'-CCGGCCATATTCACATAA-3'	*papC*	501	(2003)

PCR conditions: PCR was performed in 0.2 mm thin walled PCR tube. Each PCR tube contained 2.5 µl DNA sample and 22.5 µl aliquot of mastermix (10x PCR buffer (pH 8.3) 2.5 µl ; 50mM $MgCl_2$, 2 µl; Forward primers, 1 µl ; Reverse primers, 1µl ; Taq polymerase (3 Unit), 0.66 µl; 10 mM dNTP premix, 1 µl; Templete, 5 µl and MilliQ water, 11.84 µl.) The final reaction volume was 25 µl.

The PCR tube containing the reaction mixture was tapped thoroughly with finger and then flash spun on a microcentrifuge (Remi, India) to get reactants at the bottom. The DNA amplification reaction was performed in Thermocycler (Eppendorf Research, Germany) with a pre-heated lid. The steps and cycling conditions of thermo cycling for *papC* gene by PCR were set as Initial Denaturation at 94 °C for 3 min followed by another Denaturation at 94 °C for 30 sec, Annealing at 58 °C for 30 sec, Extension at 70 °C for 3 min and Final Extension at 72 °C for 5 min. PCR products were kept at -20°C until further analysis by agarose gel electrophoresis. Steps 2 to 4 were set for 30 cycles

Agarose Gel Electrophoresis:

The PCR product was analyzed by submersive agarose gel electrophoresis to resolve the amplified DNA fragment of the target gene.

RESULTS AND DISCUSSION:

Total twenty nine isolates of *E. coli*, recovered from buffalo meat were obtained from department and the PCR assay was standardized for the individual detection of virulence-associated genes of *E. coli* namely, *papC,* which encode for p-fimbriae. The primer sets for the gene allowed amplification of 501 bp PCR product represented by a single band in the corresponding region of the 100 bp DNA marker ladder.

An amplification of 501 bp of *papC* gene was obtained from *E. coli* isolates in the present study as reported by Ewers *et al.* (2003). The *papC* gene was obtained is 23 (79.3%) isolates out of total 29 isolates of *E. coli*, subjected to PCR. Contrary to the present report, various authors reported much less percentage of *papC* gene in *E. coli* isolates (Ananias and Yano (2008) reported that 35-65 % *E. coli* isolates were positive for *papC.* Similarly, Mora *et al*. (2007) recorded 36%, Rocha *et al.* (2008) 23.4 %, Badri *et al*. (2009) 37% and Siqueira *et al.* (2009) 23.5 %.)

E. coli isolates belonging to 12 serogroups O12, O24, O35, O60, O69, O88, O114, O139, O159, O168, O169, O172 and rough were positive for *papC* gene.

One of the best-understood gram-negative bacterial secretion pathways is the chaperone/usher pathway responsible for biogenesis of a superfamily of surface structures associated with pathogenesis. *PapD* and *papC* are the chaperone and usher for P pili of *E. coli*, respectively (David *et al*., 2002).

Specificity of single-plex PCR :

Specificity of all the primer sets employed for individual detection of virulence associated genes

papC of *E. coli* was evaluated. The PCR primer pairs used in this study were found to be specific to the virulence-associated gene i.e *papC* (501 bp) of *E. coli*. The primer was found to be specific to the target gene as it specifically amplified the designed product of that gene by PCR.

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