

# MOLECULAR CHARACTERIZATION OF VIRULENT *ESCHERICHIA COLI* ISOLATED FROM INTESTINE OF CATTLE AT SLAUGHTER HOUSE

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*A Study on Molecular Characterization of Escherichia coli isolated from the intestine of cattle collected at Slaughter house was carried out. As meat is the main source of infection spreading from animals to human, collection at slaughter house was identified for this study. Thirty five samples collected from the intestine of cattle were cultured in MacConkey, Sorbitol MacConkey and EMB agar and 10 isolates of E.coli were obtained. The isolates were confirmed based on biochemical characterization and subjected to plasmid analysis and PCR. Plasmid isolation was done by alkaline lysis method and plasmid bands were detected in 4 isolates. Multiplex PCR was carried out for the 10 isolates using primers for the virulent genes iss and fimC and both the virulent genes iss and fimC were amplified in 5 isolates.*

**Key words:** E.coli, Plasmid analysis, Multiplex PCR, Virulent genes-*fimC* and *iss*

*Escherichia coli* was first discovered by German pediatrician and bacteriologist Theodor Escherich in 1885. The organism is a Gram negative, straight rod measuring 1-3 x 0.4 -0.7 µm arranged singly or in pairs and belongs to the family Enterobacteriaceae. It is non spore forming and motile by peritrichate flagella, though some strains may be non motile. Capsules and fimbriae are found in some strains. It is an aerobe and facultatively anaerobic. Strains of *E.coli* are characterized by serological identification of somatic O, flagellar H, capsular K and fimbrial F antigen. The expression of these different antigens on bacterial cell wall results in hundreds of serologically distinct *E.coli* serotypes. K antigen is the acidic polysaccharide antigen located in the envelope which afford

protection against phagocytosis. The somatic lipopolysaccharide surface O antigen exert endotoxic activity.

## **Virulence Properties:**

Enteric *Escherichia coli* (EC) is classified on the basis of serological characteristics and virulence properties as follows:  
Enterotoxigenic *E.coli* (ETEC)  
Enteropathogenic *E.coli* (EPEC)  
Enteroinvasive *E.coli* (EIEC)  
Enterohaemorrhagic *E.coli* (EHEC)  
Enterotoxigenic *E.coli* (EAEC)

This study is under taken with the objectives of “Isolation and identification of *Escherichia coli* of enteric origin” and “ Molecular characterization

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of the *E.coli* isolates by Plasmid analysis” and Polymerase chain reaction (PCR) of the *E.coli* isolates .

***Escherichia coli* isolates:**

Intestinal swabs were collected from 35 beef cattle at slaughter house, Perambur, Chennai. Immediately after slaughter, between September and October 2007. The swabs were brought in Stuart’s transport medium to the laboratory for analysis.

The swabs were streaked onto MacConkey agar, sorbital agar and EMB agar and incubated overnight at 37° C. Fine individual lactose-fermenting colonies from each MacConkey plate were confirmed as *E.coli* isolates, based on biochemical tests, including production of indole. The *E.coli* isolates were also diffrenciated by using enterobacteriaceae kit(Hi media)

A total of 10 *E.coli* strains, isolated out of

35 samples, were subjected to Plasmid analysis and Multiplex PCR.

**PLASMID ANALYSIS:**

Plasmid isolation was done by Alkaline lysis method of Maniatis *et al.*, (1982)

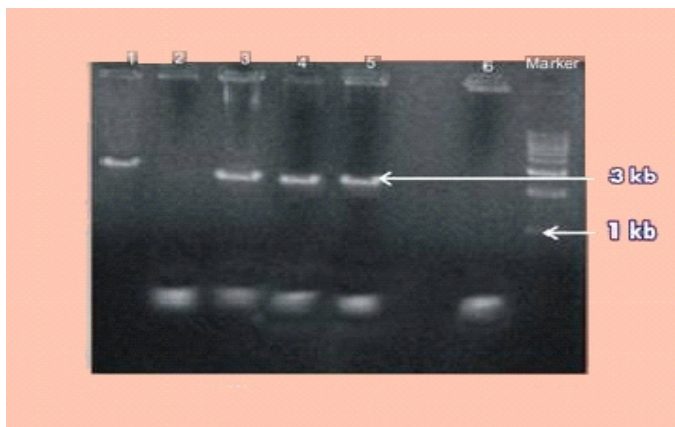
Plasmid analysis revealed the presence of bands in four isolates with the size of 3 kb in three isolates and 4 kb in one isolate. (Fig.1)

**MULTIPLEX PCR:**

Multiplex PCR was carried out following the method of Traute JanBen *et al.*, (2001)

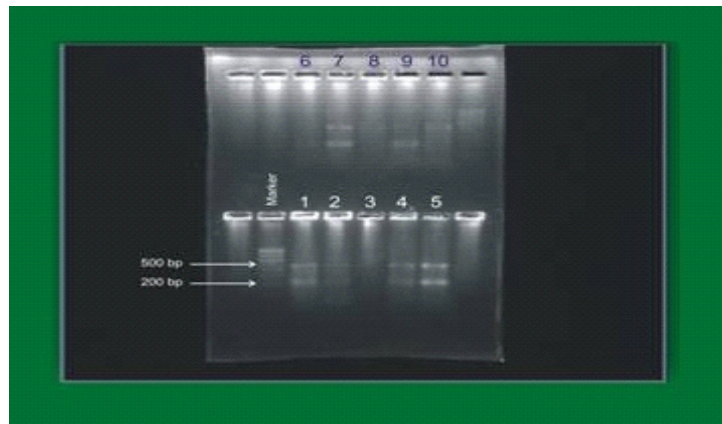
Multiplex PCR revealed the presence of virulent genes *iss* and *fimC* in five isolates, with the amplified product size of 500bp for *iss* gene and 200bp for *fimC* gene.

**Fig.1**  
**PLASMID PROFILE OF *E.COLI* isolates**



**Fig.2**

**MULTIPLEX PCR OF *E. COLI* isolates**



In this study, samples were collected from intestine of healthy cattle that were slaughtered for meat. Four *E.coli* isolates revealed the presence of plasmids, ranging in size between 3 kb and 4 kb. The results were in accordance with Uckun S.Ucan *et al.*,(2005). Five *E.coli* isolates possessed virulent genes “*iss*” and “*fimC*” accounting to 14.3% of the isolates. This finding correlates with the findings of Mc Peake *et al.*,(2005). This study reveals that *fimC* gene and *iss* gene are present in similar level in the isolates of both animals and birds. Thus Multiplex PCR is a reliable tool for molecular characterization of *iss* and *fimC* gene.

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