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PCR based detection and analysis of virulence genes of *Escherichia coli*

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Abstract

Presence of seven virulence genes (*iss*, *irp2*, *iucD*, *cvaC*, *papC*, *ireA* and *eae*) in *Escherichia coli* was studied using PCR revealed presence of only five as *iss*, *irp2*, *iucD*, *cvaC* and *papC* in isolates acquired from poultry, human and calves samples. Most of the isolates contained *iss* (65.71%) gene followed by *irp2*, *cvaC*, *papC* gene each 54.28% whereas only 45.71% isolates exhibited presence of *iucD* gene. In silico analysis was carried out using program BLASTn and software ClustalW that retrieved homologous genes.

Keywords: Virulence genes, BLASTn and ClustalW

Introduction

DNA-based methods, such as PCR, are more practical, rapid, specific and sensitive and have been used to detect one or more virulence genes. Regarding the detection of several genes at once, many multiplex PCR assays have been developed and are widely used as diagnostic methods for *E. coli* isolates**.** In the present study amplification of targeted virulence genes was done by polymerase chain reaction and their *in silico* analysis was carried out using different bioinformatics tools after sequencing.

Materials and methods

Samples from 80 Poultry post mortem, 20 calves diarrhea and 20 human infants diarrhea, totaling 120 were collected and processed for the isolation of *Escherichia coli* Extraction of nucleic acid was done and checked for quality and purity (Sambrook *et al*., 1989). The obtained DNA samples were visualized under UV transilluminator. PCR reaction was carried out for observing the presence of *iss*, *irp2*, *iucD*, *cvaC*, *papC*, *ireA* and *eae* genes.

PCR products thus obtained were sequenced and subjected for homology search using Bioinformatic tool BLAST (Basic Local Allignment Search Tool) and phylogenetic relationship was determined using ClustalW software.

Results and Discussion

Out of seven studied, only five virulence genes were accounted in 35 isolates of *E. coli*. Two genes, *ireA* and *eae* were not present in any of the isolates. In most of the poultry isolates *iss* (52.38%), *irp2* (52.38%), *papC* (52.38%) and *cvaC* (61.9%) genes were very common whereas *iucD* (83.3%) gene was more common in isolate from calf diarrhea sample (Table 1). In case of human isolates *iss* and *papC* (52.36%) genes were

Table: 1 Percentage distribution of virulence gene in species wise and overall isolates.

Host / Gene	*iss*	*Irp2*	*iucD*	*cvaC*	*papC*
Poultry	52.38%	52.38%	33.3%	61.9%	52.38%
Human	75.0%	37.5%	50.0%	25.0%	62.5%
Calf	33.3%	66.0%	83.3%	66.66%	50.0%
Overall	65.71%	54.28%	45.71%	54.28%	54.28%

more common. Most of the isolates contain iss (65.71%) gene followed by *irp2*, *cvaC*, *papC* gene each 54.28% whereas only 45.71% isolates exhibited presence of *iucD* gene agreeing with Johnson *et al*. (2006).

The sequencings of different genes were obtained in FASTA format. The BLASTn analysis of sequence of *iss* gene (309 bp) for 297 bases, and of *irp2* gene (413 bp) for 396 bases showed 99% identity with the sequences retrieved. The *iucD* gene (714 bp) showed identity of 99% with the nine sequences and 96% with one sequences for 693 bases. The *cvaC* gene (679 bp) also had maximum identity of 99% with the nine and 98% with one sequences for 672 baces. The *papC* gene (205 bp) showed 100% identity with nine sequences and 99% with one for 205 bases as observed by Larkin *et al.* (2007).

The phylogenetic tree obtained showed that gene *iss* and *cvaC* was distantly related as reported by Edger (2004). Gene *irp*2 was closely related to accession number CP002167.1 and CP002797.2, whereas gene *papC* was closely related to accession number HQ165752.1 and gene *iucD* was closely related to the cluster of accession numbers AP010960.1, AF016587.1, CP001671.1, CP003034.1 and CU928164.2 might be due to close resemblance of the strain in agreement with Winarsih *et al.* (2011).

Summary

Attempt to know presence of seven virulence were made in 35 isolates of *E. coli* obtained from 120 samples of poultry, calf and human origin elucidated only five genesas *iss*, *irp2*, *iucD*, *cvaC* and *papC* but not *ireA* and *eae*. The application of BLASTn program revealed 99% similarity for most of the studied genes. Phylogenetic tree of all the genes had similarity with some *E. coli* stains.

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