PCR-Based Detection of Horizontal Gene Transfer of iucA and ipaA Virulence Genes in Clinical E. coli Isolate EPT09 from Pakistan

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Abstract: The pathogenic E. coli strains are very important public health issue, especially in developing countries. Among the dangerous aspects of these strains is the zoonosis and acquisition of new genes through horizontal gene transfer via mobile genetic elements like plasmids, viruses or transposons. These genes include Shiga toxin (Stx1, Stx2 or variants) genes. The aerobactin operon encodes iucA, B, C and D enzymes for the aerobactin synthesis and an outer membrane aerobactin receptor Iut. In some strains of E. coli and Salmonella the aerobactin genes are found on pColV and F1me plasmids, respectively. Moreover, these genes were reported to be located on chromosome in Shigella and other E. coli strains. In the present study plasmid profile assay of clinical E. coli isolate EPT09 showed three types of plasmids designated as pEPT1 (23.1kb), pEPT2 (1.3kb) and pEPT3 (0.85kb). All the clones of EPT09 showed same plasmid profile. PCR analysis showed no amplification of the virulence gene ipaA but iucA gene was detected on plasmid DNA in EPT09. The later is reported on chromosomal DNA in Shigella. So results of present study suggest that clinical E. coli isolate “EPT09” obtained virulence gene iucA through horizontal gene transfer and was incorporated into the plasmid DNA.

Key words: E. coli • Clinical isolate • Horizontal gene transfer • Pakistan

INTRODUCTION

Escherichia coli (E. coli) is mainly nonpathogenic and is a part of the normal microflora of human and animals intestinal tract [1]. However, certain strains have acquired genes that enable them to cause intestinal or extraintestinal diseases [2]. These genes include Shiga toxin (Stx1, Stx2 or variants) genes [3, 4]. Stx1 and Stx2 are encoded by alleles in the genome of temperate, lambdoid bacteriophages [5, 6]), which appear to regulate Shiga toxin expression as part of their lytic switch [7]. There are many examples of bacterial and archaeal lineages having history of horizontal gene transfer and loss [8]. The ability of the most pathogenic bacteria to acquire iron is associated with their virulence. They use host iron sources via siderophore-mediated uptake system. Mostly, clinical E. coli isolates produce catechol siderophore enterobactin, while in some cases hydroxamate siderophore aerobactin is produced. The aerobactin operon encodes iucA, B, C and D enzymes for the aerobactin synthesis [9] and an outer membrane aerobactin receptor Iut [10]. In some strains of E. coli and Salmonella the aerobactin genes are found on pColV and F1me plasmids, respectively. Moreover, these genes were reported to be located on chromosome in Shigella and other E. coli strains [11, 12]. So, horizontal gene transfer is a useful tool for bacteria to acquire adaptations according to the new environment [13].

Pathogenic E. coli is very important public health concerned problem which should be characterized, especially on molecular basis, time to time. Till date no such study has been reported on Pakistani pathogenic E. coli strains. The current study was aimed to elaborate this important public health problem on molecular level. On the basis of data we suggest that the iucA virulent gene is located on plasmid DNA in clinical E. coli isolate EPT09 from Pakistan, which was reported on the chromosomal DNA in Shigella.
MATERIALS AND METHODS

**Bacterial Strain, Media and Plasmids:** Clinical *E. coli* isolate was obtained from Pakistan Institute of Medical Sciences (PIMS) Islamabad, Pakistan. The isolate was associated with diarrhea in a 35 years old male from poor sanitation region. The isolate was allowed to grow on solid as well as liquid medium in the Molecular Biology lab at University of Gujrat, Pakistan. For solid medium nutrient agar (Merk, Germany) was used, while nutrient broth (Britania, Argentina) was used for liquid medium according to the manufacturer’s instruction. Plasmid DNA isolated using ultraGene DNA purification kit (Anagen Technologies, USA), adopting the manufacturer’s protocol.

**Primers and Polymerase Chain Reaction:** Primers were designed through Primer3 (version. 0.4.0) [14] using reference sequences NC_007613.1 (*Shigella boydii* Sb227) and NC_007607.1 (*Shigella dysenteriae* Sd197) from National Center for Biotechnology Information (NCBI) nucleotide data base (Table 1). PCR was performed using UniEquip (Germany) thermal cycler. Thirty cycles were carried out for each PCR process and conditions were as follow: denaturation at 94 °C for 55 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 2 minutes. Initial denaturation final extension was carried out at 94°C for 3 minutes and 72°C for 10 minutes, respectively.

**Gel Electrophoresis:** The 0.8% agarose gel was used for plasmid DNA resolution, while PCR products were resolved on 1% agarose gel. To estimate plasmid DNA size λ-HindIII and 1kb DNA markers (Fermentas) were used. Similarly 100bp DNA markers were used for PCR products size. Gel results were analyzed and documented through gel documentation system (Cleaver, Germany).

- **Table 1: Primers for iucA and ipaA gene**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name/ type</th>
<th>Primer sequence (5’-3’)</th>
<th>Length (nucleotides)</th>
<th>GC%</th>
<th>Tm(C°)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iucA</td>
<td>lu1S</td>
<td>GTGGCAGGGGGAATATCTTT</td>
<td>20</td>
<td>50</td>
<td>60</td>
<td>856</td>
</tr>
<tr>
<td></td>
<td>lu1AS</td>
<td>TACACGAGCCCATCAGATTCG</td>
<td>20</td>
<td>50</td>
<td>59.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lu2S</td>
<td>ATCGCTCCATTTTCAGAGTGG</td>
<td>20</td>
<td>50</td>
<td>60</td>
<td>760</td>
</tr>
<tr>
<td></td>
<td>lu2AS</td>
<td>CATCCCACTCTCCACTCTCTTT</td>
<td>20</td>
<td>50</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>ipaA</td>
<td>lp1S</td>
<td>TGGGGAGTCATCCTTGGGATA</td>
<td>20</td>
<td>50</td>
<td>60</td>
<td>782</td>
</tr>
<tr>
<td></td>
<td>lp1AS</td>
<td>GGGCACTTCCACATCATC</td>
<td>20</td>
<td>45</td>
<td>59.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lp2S</td>
<td>TGCCCGGAAGAGAGAGTT</td>
<td>20</td>
<td>50</td>
<td>59</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>lp2AS</td>
<td>TGTCGACAGTATCGGAAC</td>
<td>20</td>
<td>50</td>
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</table>

**RESULTS**

Plasmid profile study and molecular characterization through PCR of Clinical *E. coli* isolate EPT09 was carried out. The six clones of EPT09, denoted as C-1 to C-6, were subjected to plasmid profiling. Only two representative clones C-1 and C-2 were selected for PCR-based detection of *ipaA* and *iucA* virulence genes.

The plasmid profile assay of the EPT09 showed three types of plasmids designated as pEPT1 (23.1kb), pEPT2 (1.3kb) and pEPT3 (0.85kb). All the clones of EPT09 showed same plasmid profile (Figure 1).

To study the existence of *ipaA* and *iucA* genes on the EPT09 plasmid C-1 and C-2 clones were proceeded for gene amplification by polymerase chain reaction (PCR). The EPT09 plasmid DNA was used as template for PCR to analyze the existence the virulence genes on plasmid DNA. Two primer pairs were used to amplify each gene. There was no amplification of *ipa* gene while, 760 bp fragment of *iucA* gene was amplified from C-2 clone with primer pair iu2: sense primer-lu2S

![Fig. 1: Plasmid profile of EPT09. Clones were denoted as C-1-C-6. Plasmids – pEPT1=23.1kb, pEPT2=1.3kb and pEPT3= 0.85 kb](image-url)
ipaA and iucA virulence genes amplification of C-1 and C-2 clone plasmid DNA using ip (for ipaA) and iu (for iucA) primers; M= 100bp DNA marker

Fig. 2: Reproducibility of iucA amplification of C-1 and C-2 clone plasmid DNA with two primers sets

(5’ ATCGCTCCATTTCAGAGTGG 3’) and antisense primer- Iu2AS (5’ CATCCCACGCTTCACTTCTT 3’) (Figure 2; Table 1).

For results reproducibility and to check sensitivity of PCR-based detection system for iucA gene, further C-1 and C-2 clones were subjected to iucA gene amplification through primer sets iu2, as mentioned above and iu1: sense primer- Iu1S (5’ GTGGCAGGGGAATATCTTT 3’) and antisense primer- Iu1AS (5’ TACAGAGCCAT CAGATTCG 3’) (Table 1). Of the two primer pairs, iu2 was shown to be specific and more sensitive that amplified 760 bp fragments from both C-1 and C-2 clones while, iu1 primers set amplified 856 bp fragment from only C-2 clone (Figure 3).

DISCUSSION

Shiga toxin-producing E. coli (STEC) strains, especially zoonotic, have been reported in nature [15, 16] which contained Shiga toxin-encoding genes in Pathogenicity Island on chromosomes. The pathogenicity island can be transmitted horizontally [17].

In this study we characterized clinical E. coli isolate EPT09 through plasmid profile and PCR for iucA and ipaA gene presence. Three types of plasmids were observed in EPT09 isolate. The size of the largest plasmid pEPT1 was 23.1kb and the smallest plasmid pEPT3 was 0.85kb in size. Four plasmids, ranging size 3kb to more than 90kb, were reported in the Shiga toxin-producing E. coli strains isolated from hemolytic-uremic syndrome patients, cattle and food samples [18]. The pEPT1 was identical in size to R plasmid found in Aeromonas isolated from the ulcers of epizootic ulcerative syndrome affected fish which was easily transferred to E. coli DH5α strain [19]. Ruminants, especially cattle, were reported as a vast reservoir of Shiga toxin-producing E. coli. Thus the food and water contaminated with the cattle manure caused infection in human frequently [1, 20] and meat was reported as an important vehicle for zoonotic transmission pathogenic E. coli from animals to humans [21].

The facts of bacterial genome modified by gene loss and by gene addition through duplication and the acquisition of new genes through horizontal gene transfer have been reported in a number of cases [22]. New genes acquired by horizontal gene transfer were introduced via mobile genetic elements (MGEs) such as plasmids, viruses or transposons or by direct uptake and incorporation of naked DNA by homologous or illegitimate recombination [23]. The horizontal gene transfer was also reported in Yersinia and some of the pathogenic E. coli strains, where both had high-pathogenicity islands in their chromosomes [24, 25].

Comparative genomics clearly indicates that Shigella spp. and diarrheagenic entroinvasive E. coli (EIEC) evolved from multiple E. coli strains by convergent evolution [26, 27]. The virulent gene iucA was reported in Shigella on chromosomal Pathogenicity Island (PAI) SHI-3 [28]. In Salmonella and some strains of E. coli aerobactin encoding genes, which include iucA, were reported on F1me and pColV plasmids, respectively. But in other E. coli strains these genes were found on chromosome [11, 12]. In the present study, we for the first time report the presence of iucA virulent gene on the plasmid DNA of clinical E. coli strain EPT09, with unique plasmid profile in Pakistan, which has not been reported earlier.

CONCLUSIONS

The horizontal gene transfer analysis of virulence genes iucA and ipaA through PCR showed existence of iucA gene on plasmid DNA but ipaA was not detected. This suggests the horizontal gene transfer of iucA virulence gene through plasmid to the clinical isolate of E. coli, EPT09.
REFERENCES