Commensal *E. coli* as an Important Reservoir of Resistance Encoding Genetic Elements

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**Background:** Diarrheagenic *E. coli* is the most important cause of diarrhea in children and is a public health concern in developing countries. A major public problem is acquisition and transmission of antimicrobial resistance via mobile genetic elements including plasmids, conjugative transposons, and integrons which may occur through horizontal gene transfer.

**Objectives:** The aim of this study was to investigate the distribution of class 1 and 2 integrons among commensal and enteropathogenic *E. coli* isolates and assess the role of commensal *E. coli* population as a reservoir in the acquisition and transmission of antimicrobial resistance.

**Materials and Methods:** Swabs were collected directly from stool samples of the children with diarrhea admitted to three hospitals in Tehran, Iran during July 2012 through October 2012. Antimicrobial susceptibility testing and PCR analysis were performed for analysis of the resistance pattern and integron content of isolates.

**Results:** A total of 20 enteropathogenic *E. coli* (identified as eae+stx1-stx2-) and 20 commensal *E. coli* were selected for analysis. The resistance pattern in commensal and pathogenic *E. coli* was very similar. In both groups a high rate of resistance was seen to tetracycline, streptomycin, cotrimoxazole, nalidixic acid, and minocycline. Of 20 EPEC strains, 3 strains (15%) and 1 strain (5%) had positive results for int and hep genes, respectively. Among 20 commensal, 65% (13 strains) and 10% (2 strains) had positive results for int and hep genes, respectively.

**Conclusions:** The higher rate of class 1 integron occurrence among commensal population proposes the commensal intestinal organisms as a potential reservoir of mobile resistance gene elements which could transfer the resistance gene cassettes to other pathogenic and/or nonpathogenic organisms in the intestinal lumen at different occasions.

**Keywords:** Enteropathogenic; *E. coli*; Commensal; Integron; Resistance

1. **Background**

Infectious diarrheal diseases are the second most cause of mortality and morbidity among infectious diseases among children less than 5 years old, with annually mortality rate of 3 million (1). The predominant organism in the normal flora of intestine, *Escherichia coli*, remains harmless in the intestinal lumen but in case of immune suppression or other situations may cause infection (2). Diarrheagenic *E. coli* is the most important cause of diarrhea in children and is a public health concern in developing countries. *E. coli* pathotypes should be differentiated from commensal *E. coli* in intestinal flora (3). A major public health problem is acquisition and transmission of antimicrobial resistance via mobile genetic elements including plasmids, conjugative transposons, and integrons which may occur through horizontal gene transfer (4-6). Integrons are genetic elements containing site specific for recombination, capture and mobilization of gene cassettes (7). Gene cassettes which are small non-replicating mobile DNA elements share similar structure in spite of various ranges of genes. Gene cassettes differ to a great extent in length from 262 to 1549 bp due to differences in sizes of genes they transport. Mobilized integrons contribute considerably to spread of antibiotic resistance genes (8). They are common genetic elements among both nosocomial and community of gram negative isolates (9-11). Integrons play a chief role in the arrest and expression of exogenous genetic material. So far, nine classes of integrons have been described and based on the integrase gene sequences, three classes of integrons were categorized which are responsible for multi-

**Implication for health policy/practice/research/medical education:**

The higher rate of class 1 integron occurrence among commensal population proposes the commensal intestinal organisms as a potential reservoir of mobile resistance gene elements which could transfer the resistance gene cassettes to other pathogenic and/or nonpathogenic organisms in the intestinal lumen at different occasions.

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drug resistance. Classes 1 and 2 are most frequently associated with resistance in clinical infection (12-14). A high prevalence of class 1 and class 2 integrons in Gram-negative clinical isolates have been reported from different countries. According to these data integrons are comparatively prevalent, especially in Enterobacteriaceae and play role to the spread of antimicrobial drug resistance in healthcare settings (15). It has presumptive but defective integrase gene intI2 since the nucleotide sequence is abrupt by an internal stop codon (TTA) at amino acid position 179. The intI2 gene is placed 5’ to the first gene cassette and its gene product was found to share 46% homology with intI1 (16).

2. Objectives

The aim of this study was to investigate the distribution of classes 1 and 2 integrons among commensal and enteropathogenic E. coli isolates and assess the role of commensal population as a reservoir in the acquisition and transmission of antimicrobial resistance.

3. Materials and Method

3.1. Specimens and Culture Process

Swabs were collected directly from stool samples of the children with diarrhea admitted to three hospitals in Tehran, Iran during July 2012 through October 2012. Patients were under five years of age who did not receive any antibiotics. Samples were suspended into Cary-Blair transport media and transported to laboratory on ice where one loop from each sample was streaked directly on MacConkey agar within 4 hours after collection. Plates were incubated at 37 °C for 18–24 h, and up to 5 colonies with typical appearance of E. coli were selected and subjected to biochemical tests including Oxidase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation.

3.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of EPEC isolates was determined using the disk diffusion protocols on Mueller-Hinton agar, as described by the CLSI guidelines. Each isolate was tested for susceptibility to 8 antimicrobial agents: Augmentin (25 mg), tetracycline (30 mg), streptomycin (10 mg), ciprofloxacin (5 mg), gentamicin (10 mg), ceftriaxone (25 mg), nalidixic acid (30 mg), and minocycline (30 mg) (Difco Laboratories, Detroit, USA).

3.3. Molecular Diagnostic Methods for EPEC

DNA from purified and confirmed E.coli isolates was subjected to PCR analysis (Table 1). PCR is performed in a reaction mixture with a total volume of 25 μL, containing 15.9 μL of sterile water, 2.5 μL of 10× Taq polymerase buffer, 0.3 μL of dNTPs (10 mmol/L), 1 U of Taq DNA polymerase, and 25 pmol of each primer. Amplification was performed as follows: initial denaturation step at 94 °C for 5 min, followed by 30 cycles including denaturation (94 °C for 1 min), annealing (58 °C for 1 min, separately adjusted for each set of primer pairs), and extension (72 °C for 1 min), followed by a final extension step at 72 °C for 5 min.

3.4. Detection of Class 1 and 2 Integron Conserved Regions by PCR

Presence of classes 1 and 2 integrons among the EPEC isolates was investigated using intI-F and intI-R primers for class 1 and hep-F, and hep-R for class 2 integron. The primers were specifically chosen to amplify the conserved region of each class of integron (Table 1).

Table 1. Primers Sequences Used in This Study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Amplicon Size bp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eae-F/Eae-R</td>
<td>TGGGCGACAAACGGGCGGA/CAGTCGGCCGCAACAGGATTCC</td>
<td>422</td>
<td>(17)</td>
</tr>
<tr>
<td>stx1-F/stx1-R</td>
<td>CAGTAAATGTGCTGGGGAAG/CAGTAAATGTGCTGGGGAAG</td>
<td>894</td>
<td>(18)</td>
</tr>
<tr>
<td>stx2-F/stx2-R</td>
<td>CTTCGGATCTTACATTCGG/CGATGCATCTTGGCTGATTG</td>
<td>478</td>
<td>(18)</td>
</tr>
<tr>
<td>IntI-F/IntI-R</td>
<td>TGGGTGATATCATCTGCTGTA/CAAGGATTCTGACGATGTC</td>
<td>900</td>
<td>(05)</td>
</tr>
<tr>
<td>Hep-F/Hep-R</td>
<td>CGGGATCCCAGCGCGATCCATG-CAGATTAGA/GATCCATCGCAAGTAGAG</td>
<td>variable</td>
<td>(19)</td>
</tr>
</tbody>
</table>

4. Results

A total of 20 enteropathogenic E.coli (identified as eae+stx1-stx2-) and 20 commensal E.coli collected during July 2012 and October 2012 were selected for analysis. An amplification band of 422 bp, 894 bp, and 478 bp was obtained for eae, stx1, and stx2, respectively. The result of PCR
analyses for each pair of primer sets is shown in Figure 1.

![Image](88x548 to 297x706)

![Image](88x400 to 297x540)

![Image](88x247 to 297x388)

Figure 1. A) PCR amplification of eae gene. Lanes 1-4: presumptive EPEC isolated from patients, lane 5: Positive control, lane 6: negative control. B) PCR amplification of class 1 integron integrase gene (int). Lane 1: Positive control, lane 2: negative control, lanes 3 and 4: isolates under study. C) PCR amplification of class 2 integron gene (hep). Lane 1: Positive control, lane 2: negative control, lanes 3-6: isolates under study.

4.1. Antibiotics Resistance Pattern

The antibiotic resistance pattern of isolates is shown in the Table 2. The resistance pattern in commensal and pathogenic E.coli was very similar. In both groups a high rate of resistance was seen to tetracycline, streptomycin, cotrimoxazole, nalidixic acid, and minocycline. The most dominant resistance was seen to cotrimoxazole among commensal isolates (75%, 15 isolates), while the dominant resistance among EPEC isolates was seen to tetracycline (65%, 13 isolates).

| Table 2. Antibiotics Resistance Profiles
  | Antimicrobial Agents | Resistance (%) in Commensal E. coli | Resistance (%) in Pathogenic E. coli |
  | Augmentin | 25 | 20 |
  | Tetracycline | 65 | 65 |
  | Streptomycin | 40 | 45 |
  | Ciprofloxacin | 10 | 20 |
  | Gentamicin | 15 | 5 |
  | Cotrimoxazole | 75 | 60 |
  | Nalidixic acid | 50 | 60 |
  | Minocycline | 60 | 40 |

4.2. Distribution of Classes 1 and 2 Integrons and Sizing of Class 1 Integron

Of 20 EPEC strains, 3 strains (15%) and 1 strain (5%) had positive results for int and hep genes, respectively. An amplification band of 900 bp was obtained for all int+ isolates; while, a fragment of 2300bp was obtained for hep gene among EPEC. Among 20 commensal, 65% (13 strains) and 10% (2 strains) had positive results for int and hep genes, respectively. Two fragments of 1500bp and 2300bp were obtained for hep gene among commensals.

5. Discussion

EPEC is one of the major agents of acute diarrhea among children in Iran (17). Furthermore, the emergence and spread of antimicrobial resistant E. coli and other pathogenic bacteria have become as serious public health threats. Water contaminated with effluents from livestock farms, aquaculture, hospitals, municipal wastewater treatment, or pharmaceutical manufacturing can be enriched for enteric bacteria resistance to one or more antibiotics. The distribution of class 1 was 15% and 65% among our EPEC and commensal population, respectively; Furthermore, the distribution of class 2 integron were 5% and 10% among the two groups, respectively. The higher rate of class 1 integron occurrence among commensal population proposes the commensal intestinal organisms as a potential reservoir of mobile resistance gene elements which could transfer the resistance gene cassettes to other pathogenic and/or nonpathogenic organisms in the intestinal lumen at different occasions.

Both EPEC and commensal samples showed antibiotic resistance against 8 antibiotics tested which provides an evidence for exchange of resistance genetic elements among nonpathogenic commensal E.coli and EPEC.
through horizontal mechanisms via transposons or plasmids.

The prevalence of class 1 integrons in E. coli was 49% in selected nonoutbreak isolates from hospitalized patients from 2002 to 2004 in Mexico City (20). A study performed on the prevalence and diversity of integrons and associated resistance genes in fecal E. coli isolates of healthy humans in Spain, found that integrases were associated with class 1 and/or class 2 integrons conferring resistance to a wide spectrum of antibiotics in 28% of the E. coli isolates (14). This leads to a worrisome speculation that individuals in the community could be a reservoir of integron-containing E. coli isolates and improper sanitation could easily cause the rapid dissemination of these resistant strains. Highly resistant E. coli strains are not only found in humans with disease but have also been reported in the feces of healthy humans. All these reports draw our attention to the presence of antibiotic resistance in E. coli from a wide range of sources due to integrons. Most of the strains under study were resistant to all tested antimicrobial agents except gentamicin. The result of this study is consistent with the previous results from Iran and other countries (21). One probable explanation for the high prevalence of resistance to antibiotics tested is using most of these antibiotics in conventional dairies which facilitates the natural selection and spread of resistance strains containing integrons (8).

In a study by Koczura et al. (21), four virulence encoding genes were associated with the presence of class 1 integrons; whereas, none of the genes investigated occurred in the int-nl-negative isolates. This suggests that at least in some cases, those virulence genes were localized within the same plasmids as the integrons which may reflect pathogenic potential of integron-bearing strains (21). This emphasizes on the need to monitor the distribution and content of different classes of integrons not only for their resistance but also for virulence potential. As our results indicated, integrons were present in 20% of EPEC and 70% of commensal E. coli strains. The occurrence of integron-negative antibiotic resistant strains emphasizes on multidisciplinary mechanisms of resistance among E. coli isolates of both pathogenic and commensal origins.

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Authors' Contribution

The entire manuscript was prepared by the author.

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References