Int J Entric Pathog. 2013 November; 1(2): 44-8. DOI: 10.17795/ijep13516

Published Online 2013 November 1. **Research Article**

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

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Received: July 10, 2013; **Revised:** July 23, 2013; **Accepted:** August 23, 2013

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:
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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 which 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), cotrimoxazole (25mg), 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). EPEC strains were recognized by the presence of *eaeA* gene and the absence of Shiga toxin genes (stx1, 2). PCR was 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 *int1*-F and *int1* -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

Primer	Sequence (5'-3')	Amplicon Size bp	Reference
***Eae*-F/*Eae*-R**	TGCGGCACAACAGGCGGCGA/ CGGTCGCCGCACCAGGATTC	422	(17)
***stx*1-F/*stx*1-R**	CAGTTAATGTGGTGGCGAAG/ CAGTTAATGTGGTGGCGAAG	894	(18)
***stx*2-F/*stx*2-R**	CTTCGGTATCCTATTCCCGG/ GGATGCATCTCTGGTCATTG	478	(18)
***Int*1-F/*Int*1-R**	TGCGTGTAAATCATCGTCGT/ CAAGGTTCTGGACAGTTGC	900	(05)
***Hep*-F/*Hep*-R**	CGGGATCCCGGACGGCATG-CACGATTTGTA/GATGCCATCG-CAAGTAGAG	variable	(19)

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 422bp, 894bp and 478bp was obtained for eae, *stx1* and *stx2*, respectively. The result of PCR

analyses for each pair of primer sets is shown in Figure 1.

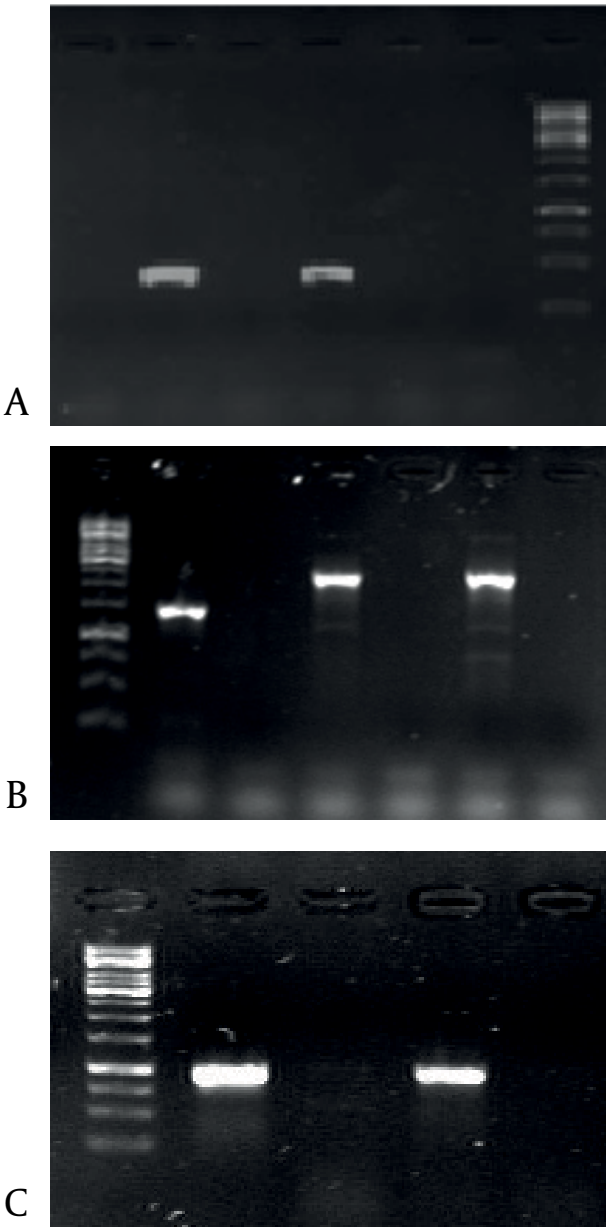

Figure 1. A) PCR amplification of eae gene. Lanes 1-4: presumptive EPEC isolated from patients, lane 5: Positive control, Lane 6: negative control. B) PCR amplification of class 1 integron integrase gene (*int*). Lane 1: Positive control, lane 2: negative control, lanes 3 and 4: isolates under study. 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
Ciprofloxacine	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 29% 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 *intI*1-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*. The occurrence of integron-negative antibiotic resistant strains emphasizes on multidisciplinary mechanisms of resistance among *E.coli* isolates of both pathogenic and commensal origins.

Acknowledgements

We thank the research council of Tarbiat Modares University for supporting the project.

Authors' Contribution

The entire manuscript was prepared by the author.

Financial Disclosure

There is no financial disclosure.

Funding/Support

The study is self-funded.

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