Prevalence of qnr, intl, and intII genes in extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli isolated from clinical samples in Iran

Morteza Hadizadeh1, Amin Norouzi2, Rohollah Taghadosi3, Samane Mohebi2, Mohsen Mohammadi4, Amir Hasanzadeh5 and Majid Taati Moghadam2*

1Department of Agriculture, Payame Noor University, Tehran, 2Department of Microbiology, Kerman Medical Students Research Committee, 3Department of Microbiology, Azalipour School of Medicine, Kerman University of Medical Sciences, Kerman, 4Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, 5Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

*For correspondence: Email: majidtaati1367@yahoo.com; Tel: +98-911-934-3489

Received: 6 July 2016 Revised accepted: 12 December 2016

Abstract

Purpose: To investigate the prevalence of qnr, intl, and intII genes in extended spectrum beta-lactamase (ESBL)-producing Escherichia coli isolated from clinical samples in Kerman, Iran.

Methods: A total of 127 E. coli were collected from clinical samples in Kerman hospitals. The antibiotic susceptibility test was performed using disc diffusion method, while the presence of ESBL-producing E. coli was determined by phenotypic confirmatory test. Furthermore, the presence of qnrA, qnrB, qnrS, intl, intII, and β-lactamase-encoding genes was detected by polymerase chain reaction (PCR). Finally, the data were analyzed and associations between different genes and antibiotic resistance were evaluated.

Results: The highest and lowest rates of resistance were observed against ampicillin (72.4 %) and imipenem (2.3 %), respectively. Also, 41.7 % of the isolates produced ESBL-enzymes. The qnrS and qnr genes were detected in 6.3 and 0.78 %, respectively, of the isolates, while qnrA gene was not detected in the current study. The results revealed that 64.5 and 10.2 % of isolates carried intl and intII genes, respectively. Data analysis showed a significant association between ESBL production and class I integrin gene in E. coli isolates.

Conclusions: Due to the variation in the resistance patterns of E. coli against antibiotics in different geographical regions, antimicrobial treatments should be based on local experience. Also, the coexistence of ESBL and intI gene in the majority of E. coli isolates suggests that care should be taken in choosing antibiotic therapy.

Keywords: Extended-spectrum β-lactamase, E. coli, Integrin, Imipenem, Bacterial genes, Antibiotic resistance

INTRODUCTION

Extended-spectrum β-lactamase (ESBLs) such as E. coli and Klebsiella pneumoniae are frequently prevalent in the Enterobacteriaceae family [1,2]. In recent years, ESBL-producing E. coli has mostly been implicated in nosocomial infections [3]. ESBL-producing organisms are resistant not only to beta-lactams, but also to most other antibiotic families such as aminoglycosides and quinolones, and that results in the limitation of therapeutic options [3,4].
ESBLs are enzymes commonly derived from TEM or SHV families of enzymes [4]. CTXM is also a concern and has become increasingly prevalent in the world since 1995 [5]. The emergence of quinolone resistance among human gram-negative pathogens is also of concern [6,7].

Several mechanisms contribute to the resistance to quinolone antibiotics; plasmid-mediated quinolone resistance qnr genes are a common mechanism [8]. Although the qnr gene is associated only with low-level resistance to fluoroquinolones, the presence of the qnr gene along with chromosomal mutations can contribute to high levels of resistance. It has also been suggested that the relationship between qnr and ESBLs is co-associated with ESBL-encoding genes. High levels of quinolone resistance in clinical strains of E. coli and other ESBL-producing bacteria have been reported in various studies [9-11]. However, the majority of recent studies on ESBL genes found that ESBL genes were located on integrons. Therefore, ESBLs can be located on integron genes and easily transferred into various bacteria [12].

The prevalence of antibacterial resistance among E. coli species is an important problem. Antimicrobial resistance patterns are different in qnr and ESBL-producing species [6,7,13]. The main purpose of the current study was to detect the relationships between ESBL and qnr and between ESBL and integrons (intI and intII) to demonstrate that ESBLs are located on integrons or plasmids.

**EXPERIMENTAL**

**Ethical issues**

This work was approved by the ethical committee of Department of Infectious Diseases, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran (ref no. 1394.223). Written informed consent was obtained from the patients or a close relative. Written informed consent was obtained from the patients or their families. All stages of the studies pertaining to patients to the patients followed international guidelines for ethics and research [14].

**Bacterial isolates**

In the current study, 127 E. coli from urine, blood, wound, and other bodily fluids were collected from Kerman hospitals. All 127 E. coli were identified biochemically using standard methods including motility, indole production, negative citrate test, glucose, sucrose, and lactose fermentation in TSI agar, and positive MR reaction in MRVP medium. After E. coli was identified, the isolates were evaluated for antimicrobial resistance.

**Antimicrobial susceptibility test and β-lactamase determination**

Antibiotic susceptibility tests were carried out using the disc diffusion method according to Clinical and Laboratory Standard Institute guidelines (CLSI). Antibiotic discs including ampicillin, ceftriaxone, cefotaxime, ceftazidime, co-trimoxazole, nalidixic acid, ciprofloxacin, gentamicin, amikacin, and imipenem were used. ESBL-producing E. coli was determined using the phenotypic confirmatory test. The phenotypic test was performed using cefotaxime-clavulanic acid or ceftazidime-clavulanic acid as two-disk synergism versus ceftazidime or cefotaxime alone. If the inhibition zone around the synergism disks compared with ceftazidime or cefotaxime alone was ≥ 5mm, then these species was found to be ESBL-producing. E. coli (ATCC 25922) was used as a negative control to determine antibiotic susceptibility.

**Detection of qnr, intI, intII, and β-lactamase-encoding genes**

The presence of intI, intII, qnrA, qnrB, qnrS, bla-TEM, bla-SHV, and bla-CTXM genes was detected by standard PCR amplification. First, E. coli colonies were grown in MacConkey agar and then transferred into 1 ml of water in an Eppendorf tube to be boiled. After 15 mins boiling, DNA templates were extracted for PCR amplification. PCR reactions were performed in a final volume of 25 µL using 3 µL of DNA solution, 12.5 µL Qiagen HotStar Taq polymerase Master Mix, 10 pmol primer, and water. PCR cycling conditions were as follows: denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, extension at 72 for 40 s, and the final extension step at 72 °C for 5 min. All primers used in the current study are listed in Table 1. PCR products were detected using electrophoresis on a 1 % agarose gel. The agarose gel was stained with ethidium bromide. The agarose gel was illuminated by UV and then analyzed. Figures 1 and 2 demonstrate the locations of the genes on the agarose gel. Klebsiella pneumonia (ATCC 700603) was used as the positive control for bla-SHV and bla-CTXM genes. E. coli (ATCC 35218) was also used as the positive control for the bla-TEM gene, and E. coli (ATCC 25922) was considered as the negative control. Acinetobacter baumannii (TMU1, TMU2) were used as the positive controls for integron classes I and II.

Trop J Pharm Res, January 2017; 16(1): 142
Furthermore, *E. coli* J53 strains containing pMG252, pMG298, and pMG306 were used as *qnrA*, *qnrB*, and *qnrS*-positive controls, respectively.

**Statistical analysis**

Data was analyzed using SPSS, version 22 and is presented as mean ± standard deviation (SD). Associations between different genes and antibiotic resistance were also evaluated using the chi-square (X²) test. *P* < 0.05 was considered statistically significant.

**RESULTS**

The findings indicate that the resistance rates of ESBL-producing isolates to all antibiotics were higher than those of non-ESBL-producing isolates. In the current study, 127 *E. coli* were isolated from a range of clinical specimens including the bodily fluids of patients with urinary tract, blood, wound, and other types of infection. *E. coli* isolates were most frequently isolated from patients with urinary tract infection (89.7 %); the isolated samples of blood, wound, and bronchial fluid were 3 (2.3 %), 6 (4.7 %), and 5 (3.9 %), respectively. Of all 127 samples, 82 (64.5 %) were isolated from females compared to 45 (35.4 %) samples isolated from males. As shown in Table 2, even though imipenem was the most effective antibiotic against *E. coli* isolates, susceptibility to amikacin, ciprofloxacin, and gentamicin was also high. Ninety-two isolates (72.4 %) were resistant to ampicillin, a higher percentage than for any other antibiotic. Resistance to co-trimoxazole and nalidixic acid was also significant. *E. coli* clinical isolates showed variable susceptibility to different antibiotics. For example, isolates recovered from urinary tract infections were more resistant to ciprofloxacin and gentamicin than others, but resistance to imipenem, ampicillin, and ceftriaxone was higher in wound and bronchial fluid samples. Details of the antibiotic susceptibility tests are presented in Table 2.

The results of this study further showed that various isolates from urinary tract, blood, and wound infections as well as other bodily fluids were significantly associated with the prevalence of ESBLs (Table 3). One hundred percent and 33.6 % of *E. coli* isolates from wound and urinary tract infections were ESBLs-containing, respectively (*P* < 0.05). ESBL-producing isolates were determined using the phenotypic disk technique in which 53 (41.7 %) isolates were found to produce ESBL enzymes. After PCR detection, all phenotypically-positive isolates contained ESBL genes. The *bla-TEM* gene was the major ESBL type observed in 86.7 % of ESBL-producing isolates. Among 53 identified ESBL-producing *E. coli*, 81.1 % and 5.6 % were *bla-CTXM* and *bla-SHV*, respectively. However, the majority of ESBL-producing isolates were susceptible to imipenem (94.4 %) and amikacin (90.6 %).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence (5'-3')</th>
<th>Product size (bp)</th>
<th>Tm (č)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla-SHV</em></td>
<td>F: TCAGCGAAAAACACCTTG&lt;br&gt;R:TCCCGCGAGATAAATCACC&lt;br&gt;F:-GAGTATTCACACATTTCGGTGTC&lt;br&gt;R: TAATCAGTGAGGCACCTATCTC</td>
<td>471</td>
<td>56</td>
<td>[15]</td>
</tr>
<tr>
<td><em>bla-TEM</em></td>
<td>F:-GAGTATTCACACATTTCGGTGTC&lt;br&gt;R: TAATCAGTGAGGCACCTATCTC</td>
<td>861</td>
<td>59</td>
<td>[15]</td>
</tr>
<tr>
<td><em>bla-CTXM</em></td>
<td>F:-CGCTTTTGCAGATGTGCAG&lt;br&gt;R: ACCCGCGATATCGTTGGT</td>
<td>550</td>
<td>59</td>
<td>[16]</td>
</tr>
<tr>
<td><em>qnrA</em></td>
<td>F: GATCGGCAAAGGTTAGGTCA&lt;br&gt;R: ATTTCTCAGCCGAGATTGTG</td>
<td>627</td>
<td>53</td>
<td>[17]</td>
</tr>
<tr>
<td><em>qnrB</em></td>
<td>F: GATCGTGAAGCCAGAAGGG&lt;br&gt;R: ACAGATGCTGGTAGTTGTC</td>
<td>562</td>
<td>53</td>
<td>[17]</td>
</tr>
<tr>
<td><em>qFnrS</em></td>
<td>F: AGTGATCTCACTTACCCG&lt;br&gt;R: CAGGCTGCAATTTCG ATACC</td>
<td>550</td>
<td>53</td>
<td>[17]</td>
</tr>
<tr>
<td><em>intI</em></td>
<td>F: CAGTGAGCAACACACCTTCTC&lt;br&gt;R: CCCGAGCCATAGACTGTA</td>
<td>160</td>
<td>55</td>
<td>[18]</td>
</tr>
<tr>
<td><em>intII</em></td>
<td>F: TTGCCGAGTATCTCTACCTG&lt;br&gt;R: TTACCTGCACTGATATTAC</td>
<td>789</td>
<td>55</td>
<td>[18]</td>
</tr>
</tbody>
</table>
Antibiotic susceptibility results also demonstrated that antibiotic resistance patterns of ESBL-producing *E. coli* are different from non-ESBL ones. ESBL-positive *E. coli* showed high level resistance (>80%) to third-generation cephalosporins, but resistance to third-generation cephalosporins in ESBL-negative *E. coli* was low. Furthermore, resistance to fluoroquinolone antibiotics was higher in ESBL-positive *E. coli* than in ESBL-negative *E. coli*. In this study, a direct relationship between the presence of *bla-CTXM* and *bla-TEM* genes in *E. coli* and resistance to some antibiotics such as gentamicin, ciprofloxacin, ampicillin, ceftriaxone, cefazidime, cefotaxime, imipenem, and co-trimoxazole were observed (*P < 0.05*). Isolates producing *bla-CTXM* were more resistant than those producing *bla-TEM* and *bla-SHV*. For example, resistance to ciprofloxacin was higher in *bla-CTXM*-producing isolates than in the others (*p < 0.05*).

Overall, the isolates were screened for the presence of the *qnr* gene by PCR; 8 (7.2%) isolates containing this gene were observed. *qnrS* was the most dominant gene among the *qnr* genes. Of the 8 *qnr*-producing *E. coli* isolates, only one isolate produced *qnrB*, but *qnrS* was detected in all of the 8 isolates. No *qnrA* gene was identified in this study. After analysis, the data showed that 3 (37.5%) *qnr*-producing isolates contained ESBL genes as well, but 5 (62.5%) isolates were without these genes. The *qnr*-producing isolates were found in those *E. coli*-producing ESBLs that had both *bla-TEM* and *bla-CTXM* genes, but they were not found in *bla-SHV*-producing *E. coli*.

Based on data analysis, there was no significant association between ESBL and the *qnr* gene. Interestingly, the prevalence of the *intI* gene was observed in the majority of isolates such that 82 (64.5%) isolates presented this gene, but only 13 (10.2%) of the 127 *E. coli* isolates carried the *intI* gene. Class I integrons were more common in ESBL isolates than in non-ESBL isolates. In this study, a significant association between ESBL and integron class I was observed (*P < 0.05*). Conversely, no association was found between integron class II and ESBL genes.

![Figure 1: Agarose gel electrophoresis of representative *intI* gene detected in *E. coli* isolates. Note: DNA ladder was consist of size marker range 100 - 1500 bp. 1 = ladder, 2 = positive control, 3 = negative control. Other numbers indicate the isolates showing *intI* gene](image)

Table 2: Rate of antibiotic susceptibility (%) in ESBL-positive, ESBL-negative, and total *E. coli* isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>ESBL-positive</th>
<th>ESBL-negative</th>
<th>Samples</th>
<th>Resistance</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>96.2</td>
<td>55.40</td>
<td>72.40</td>
<td>2.30</td>
<td>25.10</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>81.1</td>
<td>18.90</td>
<td>40.90</td>
<td>0.70</td>
<td>58.20</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>84.9</td>
<td>5.40</td>
<td>42.01</td>
<td>3.30</td>
<td>54.60</td>
<td></td>
</tr>
<tr>
<td>Cefazidime</td>
<td>92.4</td>
<td>4.00</td>
<td>31.80</td>
<td>8.60</td>
<td>59.40</td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>95.5</td>
<td>54.04</td>
<td>70.80</td>
<td>2.60</td>
<td>26.70</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>80.0</td>
<td>28.0</td>
<td>51.10</td>
<td>3.10</td>
<td>45.60</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>54.6</td>
<td>6.70</td>
<td>26.90</td>
<td>3.90</td>
<td>69.04</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>33.9</td>
<td>1.30</td>
<td>15.70</td>
<td>2.30</td>
<td>81.40</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>9.40</td>
<td>5.40</td>
<td>7.08</td>
<td>8.60</td>
<td>84.20</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>5.60</td>
<td>0.00</td>
<td>2.30</td>
<td>2.40</td>
<td>95.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Relationship between integron Classes I and II with ESBLs and non-ESBLs for various specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Integron Class I</th>
<th>Integron Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>ESBLs</td>
</tr>
<tr>
<td>Urine</td>
<td>73</td>
<td>29</td>
</tr>
<tr>
<td>Wound</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Blood</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fluid</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Trop J Pharm Res, January 2017; 16(1): 144*
DISCUSSION

In the current study, ESBL-producing *E. coli* was found to be more than 40% in the clinical samples. In addition, the results suggest the emergence of a high frequency of ESBLs in clinical isolates, which may have been caused by the excessive use of broad-spectrum antibiotics in hospitals. In fact, resistance to β-lactam antibiotics and cephalosporins was more common than to other antibiotics because of the lack of attention paid to antibiotic consumption. Ampicillin and co-trimoxazole had the highest percentages of resistance, but the highest percentages of susceptibility were observed for imipenem, amikacin, ciprofloxacin, and gentamicin. The current findings are in congruence with those of other studies conducted by Calbo *et al.* and Mehrgaran *et al.* [4,18].

It is noteworthy that in this study, ESBL enzymes were detected in the majority of isolates in which bla-TEM was the most common ESBL isolated from clinical samples. Furthermore, the prevalence of bla-CTXM and bla-SHV in *E. coli* isolates was high. Table 2 demonstrates that the resistance rate of ESBL-producing isolates to all antibiotics was higher than that of non-ESBL-producing ones, especially to ampicillin, co-trimoxazole, and cefazidime. These results are congruent with several studies conducted in Iran and other countries. According to a study done by Zaniani *et al.*, ESBL was found in 43.9% of isolates, demonstrating the prevalence of ESBL in *E. coli* isolates [19]. The results of Zaniani *et al.* corroborate those of the current study. Also, Calbo *et al.* demonstrated the prevalence of ESBL in *E. coli* isolates and detected TEM (60%), SHV (16%), and CTX-M (24%) were also detected in these bacteria [18].

Some studies in Iran have demonstrated that ESBLs have been increasing during recent years. For example, Pakzad *et al.* and Nakhaei Moghaddam *et al.* reported ESBL-containing *E. coli* in 28% and 33% of isolates, respectively [20,21]. Based on the results of the above-named studies, the increase in ESBL genes found in *E. coli* isolates in the last few years is a major problem. The current study found the prevalence rates of the intI and intII genes in *E. coli* isolates to be 82 (64.5%) and 13 (10.2%), respectively. These results are similar to results obtained by Elisabete Machado *et al.* [12]. Moreover, in a study by Aziz Japoni *et al.*, the existence of integrons was confirmed in 44.8% of *E. coli* isolates, and the prevalence of integrons was about half the amount obtained in the current study [22]. The prevalence of integrons was 55.5% in Mexico and 26.1% in Turkey [23,24]. These results confirm that the prevalence of integron genes varies between Iran and other parts of the world. This resistance can probably be associated with a combination of ESBL genes and integron class I genes.

Integrons play an important role in the antibiotic resistance of clinical *E. coli* strains, and they are able to capture, integrate, and express those gene cassettes encoding antibiotic resistance [12]. In this study, a significant association between ESBLs and integron class I genes was observed as it was in several other studies the results of which agree with the current results [12,25]. Therefore, it can be said that ESBLs genes are located on class I integrons. Nevertheless, 8 (7.2%) qnr-positive *E. coli* isolates were identified among a total of 127 clinical isolates. Although the prevalence of qnr genes was low, this plasmid-borne mechanism is a major concern for increasing antibiotic resistance in other isolates. There results have also been confirmed by other investigators, such as Mammeri *et al.* who reported that qnr genes were only found in 0.3% of *E. coli* isolates [13]. Similar to the current study, Jeong *et al.* demonstrated that the prevalence of qnr genes in *E. coli* isolates was low [7].

CONCLUSION

The findings of this study indicate that amikacin, imipenem, ciprofloxacin, and gentamicin are the most appropriate oral antibiotics for treating ESBL infections in Kerman, Iran, but ampicillin is not a good choice for treating *E. coli* infections.
The high prevalence of ESBL and intI1 genes in the hospital setting can cause antibiotic resistance. Since available treatment options are limited, antibiotic control policies combined with the implementation of infection control measures are of utmost importance. Furthermore, adherence to health recommendations in hospitals and appropriate prescribing of antibiotics can minimize antibiotic resistance resulting from ESBLs, integrons, and qnr-borne bacteria in the future.

DECLARATIONS

Acknowledgement

The authors are grateful to Kerman Medical Students Research Committee for financially supporting this work.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/road), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES


