Sjournals
Scientific Journals
Scientific Journal of Microbiology (2014) 3(10) 104-110
ISSN 2322-2948
doi: 10.14196/sjm.v3i10.1741

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

The prevalence of TEM gene among extended-spectrum beta-lactamase producing *Escherichia coli* in Karaj, Iran

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ARTICLE INFO

Article history,
Received 13 August 2014
Accepted 18 October 2014
Available online 29 October 2014

Keywords,
Escherichia coli
TEM
Extended-spectrum beta- lactamase

ABSTRACT

Resistance to usually used antibiotic drug therapy represents one of the most important concerns for the treatment of bacterial infections. The increasing resistance to cephalosporin and monobactam antimicrobials is due to the production of extended-spectrum beta-lactamases (ESBLs). These ESBLs enzymes evolved via point mutations of important amino acids of extended-spectrum beta-lactamases such as TEM-1, TEM-2 and SHV-1. The aim of the present study was to investigate the prevalence of TEM gene among extended-spectrum beta- lactamase producing Escherichia coli that isolated from hospitalized patients in Karaj hospitals. In this research, a total of 77 well-characterized isolates of E. coli from patents urines were selected and identified using standard microbiological screening tests and biochemical methods. , Disks containing 10 µg of clavulanic acid and 30 µg of ceftazidime or 30 µg ceftriaxone were placed on the inoculated plates containing Mueller-Hinton agar. The cultured colonies of extended-spectrum beta lactamase producing E. coli were suspended in 100 nicrolitter ddH2O and their DNA was isolated using simple boiling method.Genotypic assay of TEM gene among extended-spectrum beta- lactamase producing Escherichia coli was performed byPCR analysis. From 77 E.coli isolate samples phenotypically examination, disk diffusion method, showed that 39.7% of cases were ESBL producing samples and 60.3% were non- ESBL. Genotype examination with degenerative primers for all 119 types of TEM gene revealed that 74 (96.1) cases

were TEM positive. These results indicated the importance of TEM gene in beta-lactam antibiotics hydrolysis between E. coli strains isolated in Karaj, Iran. The identification of this gene frequency in E. coli isolates can speed up the process of diagnosis and treating of patients in clinics.

1. Introduction

Escherichia coli (E. coli) as one of the Gram negative member of the Enterobacteriaceae family are the most common frequent cause of intra-abdominal, soft tissue infections and for acquired urinary tract infections (UTIs). Therefore, resistance to usually used antimicrobials is one of the most important concerns for the treatment of bacterial infections [1].

The significant increasing resistance to oxyimino-cephalosporin and monobactam antimicrobials is due to the production of extended-spectrum b-lactamases (ESBLs) as a heterogeneous group of bacterial enzymes [1]. ESBLs genes are plasmid mediated and can spread among other bacterial cells with resulting resistant isolated to other antibiotic classes. Production of Beta-lactamase enzyme is the main causative factor for increasing bacterial resistance to different antibiotics of this class [2, 3].

In general, Enterobacteria especiallyE. coliare a main ESBLs carrier in worldwide [3]. The ESBLs enzymes have a broad spectrum profile that allows to hydrolysis of oxyimino-cephalosporins and monobactams but not cephamycins. They are usually inhibited by clavulanic acid, sulbactam and tazobactam as beta-lactamase inhibitors [4].

These ESBLs enzymes evolved via point mutations of important amino acids of extended-spectrum beta-lactamases such as TEM-1, TEM-2 and SHV-1 (4). B-lactam antibiotics are the most used therapy for microbial infections. Microorganisms producing ESBL are associated with an increase of morbidity, mortality, and then increased health care costs [4, 5].

The TEM gene was first reported in E. coli that isolated from a person named Temoniera in Greece [2]. Adequate and rapidly ESBL detection is vital for control measures of infections and the selection of anti-microbial therapy; therefore it should be instituted as soon as possible [5]. Because phenotypic detection of ESBLs is time consuming and the results may be difficult to interpret, a fast and accurate detection method is desirable. Genotypic detection of TEM and SHV ESBL mutations therefore requires identification of single nucleotide polymorphisms (SNPs) [6]. According to reported literature, there are no available results to study of prevalence of TEM gene in Iranian population. Therefore, the aim of the present study was to investigate the prevalence of TEM gene among extended-spectrum beta- lactamase producing Escherichia coli that isolated from hospitalized patients in Karaj hospitals.

2. Materials and methods

2.1. Reagents and solutions

All antibiotics, PCR materials and agarose were purchased from Fermentas Company (U.S.A.). The other reagents and chemicals with analytical and molecular grade were from Merck (Darmstadt, Germany). Also, in this study all of the required solutions were prepared in double-distilled water.

2.2. Media and culture conditions

In this study the Muller-Hinton agar plate as selective media was adjusted at pH 7.2-7.4 at room temperature and was used. The medium was sterilized by autoclaving at 121 for 20 min. All of used antibiotics were filter sterilized (30 µg/ml) and then added to the sterile medium.

2.3. Sample collection

The current study was approved by the Ethic Committee of Islamic Azad University of Karaj, Iran and patients contributed to this study signed an informed consent in accordance with the principles of the Helsinki II declaration. All patients were from Karaj province of Iran with that who admitted to the Karaj University of Medical Sciences Hospitals and related clinics during 2010 -2012. In this research, a total of 77 well-characterized isolates of E. coli from patents urines were selected and identified using standard microbiological screening tests and biochemical methods.

2.4. Phenotypic screening test for extended-spectrum beta lactamase

Extended-spectrun beta lactamase -producing isolates identification was carried out using disks containing amoxicillin (30 µg) ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), and clavulanic acid (10 µg) on Mueller-Hinton agar. Generally, disks containing 10 µg of clavulanic acid and 30 µg of ceftazidime or 30 µg ceftriaxone were placed on the inoculated plates containing Mueller-Hinton agar.

2.5. Plasmid DNA extraction

The cultured colonies of extended-spectrum beta lactamase producing E. coli were suspended in 100 nicrolitter ddH2O and their DNA was isolated using simple boiling method. The concentration and yield of extracted DNA were assessed spectrometricaly using the Nanodrop (Thermo) with measuring the absorbance at wavelength of 260 nm. Also, quality of extracted DNA was checked with the measuring the OD 260/280 ratio. Then, the probability of DNA degradation was evaluated by electrophoresis of extracted DNA on 0.1% agarose gel containing Syber safe DNA stain [7].

2.6. Genotypic analysis of TEM gene among extended-spectrum beta- lactamase producing *Escherichia coli*

Two µl of extracted DNA was used as a template for polymerase chain reaction (PCR). A 936 bp fragment was amplified with the following universal primer: 5´ kacaataaccctgrtaaatg3´and 5´ agtatatatgagtaaacttgg 3´. PCR was performed using Master Cycler Gradient-Eppendorf (Germany) with 20 pmol of each primer, 3 µl of extracted DNA, 200 µMdNTPs, 1.5 mM MgCl2, 1 U Taq polymerase and 2.5 µl of 10X PCR buffer in afinal volume of 25 µl. The PCR thermal cycling parameters were: 1 cycle at 94 oC for 3 min, followed by 35 cycles at 94 oC for 30-60 s, 52 oC for 30-60 s and 72 oC for 90 s, and thereafter the last cycle was continued for 10 min at 72 oC as final extension. The PCR product was monitored using electrophoresis on 1% agarose gel containing Syber safe DNA stain[8].

2.7. Statistical analysis

Quantitative parameters were expressed as means ±SD. Odds ratios were measured as estimates of relative risk for disease and 95% confidence intervals were measured by logistic regression by SPSS software. A two-tailed Student's t test analysis was used to compare quantitative results. Statistical significance was assumed at the $p<0.05$ level. Statistical analyses of obtained results were performed using SPSS software package version 18.0 for windows [9].

3. Results

To Detection of extended spectrum Beta- lactamase enzyme production by phenotypic method, bacterial samples were cultured in Mueller-Hinton agar, then antibiotic disks, amoxiclav (30mcg), cefotaxim(30mcg), ceftriaxone (30mcg), ceftazidime (30mcg) and ceftizoxime (30mcg), were located on the media with 20-30 mm space; after 24 hours incubation the growth inhibition zonewas evaluated (figure 1). Results showed that, 39.7 % of the samples are ESBL producing and 60.3% are non-ESBL producing.

Fig. 1. ESBL positive plate including antibiotic discs.

Also, TEM beta-lactamase gene frequency was evaluated using polymerase chain reaction, PCR, by universal primers. (Figure 2) The results of this step showed that from 77 samples, 74 (96.1%) samples were positive for TEM beta-lactamase gene and 3 (3.8%) samples from whole were negative for TEM gene.

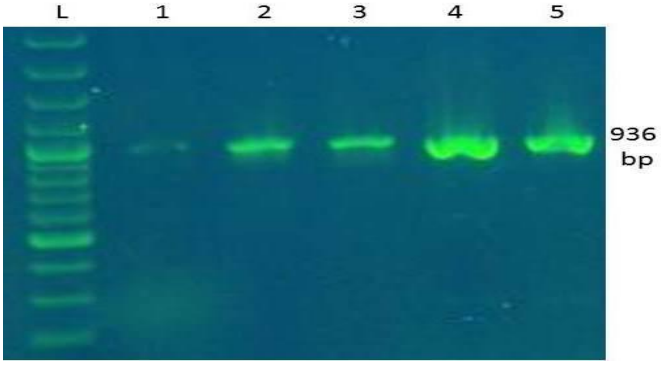

Fig. 2. 936bp PCR products of the TEM gene.

It should be mentioned, from three TEM negative samples, one case in phenotypic survey, manifested a growth zone inhibition from amoxiclav to cefotaxim (with 1.8 cm in diameter) indicative of ESBL.

4. Discussion

Beta-lactamase enzyme hydrolysis beta-lactam ring and causes degradation and inactivation of antibiotics. The introduction of third generation cephalosporins was considered as revolution in the clinical battle against bacterial resistance mediated beta-lactam producing microorganisms. These antibiotics decrease the prevalence of beta-lactamase in specific microorganisms, for instance, ampicillin hydrolysis TEM-1 and SHV-1 in the E. coli and klebsiellapneumoniae.[10, 11]TEM-1 is the most prevalent plasmid beta-lactamasegene causing resistance to ampicillin in intestinal Gram-negative bacteria such as *E.coli*, while SHV-1 is more produced by klebsiellapneumoniae.[12]Worldwide resistance to beta-lactam antibiotics of Gram negative bacteria from clinical cases has been increased [13].

ESBLs are beta-lactamase that have potential to show resistance to hydrolysis by penicillin, aztreonam, first, second and thirdgeneration of cephalosporins and also are limited by beta- lactam inhibitors such as clavulanic acid [14, 15].

The correct detection of ESBL producing microorganisms is a challenge for the laboratories, requiring not only phenotypic tests by also genotypic tests for all genes linked with beta-lactamase production.Based on the majority of epidemiological surveys on ESBL,E. coli is one of the most common species that has role in this type of resistance.[16]

The aim of this study was to evaluate the prevalence of ESBL producing E. coli and to detect the presence of TEM gene among ESBL producing isolates.

The present study was done upon 77 *E.coli* isolate samples; phenotypically examination, disk diffusion method, showed that 39.7% of cases wereextended spectrum beta- lactamase producing samples and 60.3% werenon- extended spectrum beta- lactamase producing samples. Genotype examination with degenerative primers for all 119 types of TEM gene revealed that 74 (96.1) cases were TEM positive. From 3 TEM negative cases,

phenotypically examination revealed that one case is ESBL positive; about this case there is probability of presence of SHV and/or CTX beta- lactamases.

Although some ESBLs confer high level resistance to cephalosporins, for other ESBLs, resistance may only be a little increased or selectively affected in certain beta-lactams. This causes difficulty for the clinical laboratory. Some ESBLs may fail to reach a level to be detectable by phenotypic tests but result in treatment failure in the infected person.[17] No relation between ESBL production and phenotypic results is manifest in the present study. These types of discrepancies between susceptibility data and phenotypic results have increased the need for an improved method of ESBL detection and to incorporate it into routine susceptibility procedures.

Grover et al in a study on phenotypic and genotypic methods for ESBL detection determined PCR to be a trustworthy method in comparison with phenotypic method [18].

The prevalence of ESBL productions revealed a significant geographical difference, ranging from 0% (Iceland) to less than 1% (Estonia) to 41% for E. coli (Romania).[19]In Italy, the prevalence of E. coli producers of ESBL has also increased with a predominance of TEM (45.4%). [20]

A number of previous studies have showed the high prevalence of ESBLs producing E. coli. In Asia, the prevalence of ESBLs producing E. coli isolates varies in different countries. In India, the prevalence of the E. coli isolates was reported 46.51%[21].Also, 2008 in Thailand, Kiratisin et al. have shown among 235 strains of E. coli producers of ESBLs, 77% were carrying TEM gene [22].The prevalence of the organisms in Taiwan was in the range of 1.5% to 16.7% in E. coli[23]. In Korea, the prevalence of these organisms was in the range of 4.8%-7.5% for E. coli[24]. In a study in Thailand, the prevalence of the ESBLs producing organisms in children was reported 27% in E. isolates[25]. In Pakistan, the prevalence of the ESBLs producing E. coli was reported 41% in E. coli isolates [26]. Another study in Pakistan showed that the prevalence of the ESBLs producing E. coli isolates was 56.9% [27].

Based on the results of Zaniani et al study in Iran (2012), the prevalence of ESBL producing E. coli was high (15.62%) and a total number of about 68.5% of ESBL producing isolated bacteria were TEM and/or SHV positive [2].

Reported ESBLs producing rates in E. coli isolates from various parts of Iran varies from 8.9% to 67% in E. coli isolates[2]. In our study, the prevalence of ESBLs producing E. coli was 96.1 %.

In a study that was carried out by Fazly Bazzazet al in 2007 in Mashhad, Iran, the prevalence of ESBLs producing E. coli was reported 57.5% and generally the prevalence of ESBLs producing organisms were 59.2% by Kirby-Baure disk diffusion method and the phenotypic disk confirmatory test [28].In our study, the prevalence of ESBLs producing E.coli was higher in comparison with the reported prevalence by their study.

In the Rastegar Lari study[29], ESBLs producing E. coli had 85.6% TEM that was somehow near to our results.

5. Conclusion

In summary, the prevalence of ESBLs producing E.coli in Karaj, Iran is too much high. It is necessary for health care systems being aware of ESBLs producing microorganisms. Also, the ESBLs production monitoring is recommended to avoid treatment failure and suitable infection control.

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