

Full Length Research Paper

Molecular detection of *bla TEM* and *bla SHV* genes among clinical isolates of *Escherichia coli* from Kashan, Iran

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Escherichia spp. are opportunistic pathogens that cause nosocomial infections. Because of their acquisition of multi drug resistant plasmids, these organisms are resistant to a number of antibiotics, including extended spectrum cephalosporins and aminoglycosides. The aim of this study is to detect extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from Shahid Beheshti Hospital in Kashan. This descriptive study was done on clinical specimens isolated from Shahid Beheshti Hospital in Kashan. Identification of the isolated bacteria was done by standard biochemical testes. Determination of antimicrobial susceptibility was done by disk diffusion method. The ESBL production was investigated on isolates by double disk synergy technique. ESBL producers were confirmed by MIC method. PCR amplification of ESBL genes, TEM-1 and SHV-1 was carried out. 150 specimens were *E. coli* and 70 were ESBL (46%). Of the total of 70 specimens isolates, 9 out of 40 (13%) included TEM-1 and SHV-1. 5 specimens (7%) were SHV-1 and 44 specimens (63%) were TEM-1. Given the high levels of resistance, accurate anti-biogram tests before prescribing antibiotics and avoiding indiscriminate use of antibiotics are essential.

Key words: B-lactamase, extended spectrum beta-lactamase (ESBL), *Escherichia coli*, nosocomial infections.

INTRODUCTION

With the discovery of antibiotics (Fleming, 1928), there is the emergence of new antibiotics with promotion of their use. One of the ways of resisting beta-lactam is the production of beta-lactamase enzymes, which has created many problems related to antibiotic treatment (Dalhoff, 1979). Beta-lactamases are enzymes produced by some bacteria and are responsible for bacteria resistance to beta-lactam antibiotics like penicillins, cephamycins and carbapenems (ertapenem). Cephalosporins are relatively resistant to beta-lactamase. These antibiotics have a common element in their molecular

structure: a four-atom ring known as a beta-lactam. The beta-lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties (Bush and Fisher, 2011).

In 1983, the first ESBL-producing organism was isolated in Germany. Thereafter, such organisms were reported in the United States following outbreaks of infections caused by these pathogens. In recent years, the importance of such ESBL-mediated infections has been increasingly reported worldwide (Duman et al., 2005). With the widespread and increasing use of cephalosporin antibiotics, there were groups of other beta-lactamase, with more activity spectrum compared to the initial beta-lactamase (Giamarellou, 2005).

These extended spectrum beta-lactamases (ESBLs) are: mutant, plasmid-mediated b-lactamases derived

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Table 1. PCR primers and conditions used in this study.

PCR condition	Primer	Target
95°C, 4 min; 95°C, 60 s; 50°C/35, 60 s; 72°C, 60 s; 72°C, 10 min	5'ATAAAATTCTTGAAGACGAAA3' 5'GCAAGTTACCAATGCTTAATCA3'	blaTEM-1
95°C, 4 min; 95°C, 60 s; 55°C/35, 60 s; 72°C, 60 s; 72°C, 10 min	5'TGGTTATGCGTTATATTCGCC3' 5'GGTTAGCGTTGCCAGTGCT3	blaSHV-1

from older, broad-spectrum β -lactamases (e.g., TEM-1, TEM-2, SHV-1) and have an extended substrate profile which allows hydrolysis of all cephalosporins, penicillins and aztreonam. These enzymes are most commonly produced by *Klebsiella* spp. and *Escherichia coli* (Paul et al., 1989; Marchandin et al., 1999).

The production of extended-spectrum β -lactamases (e.g., TEM-1, TEM-2 and SHV-1) by gram-negative bacteria renders ineffective all penicillins, cephalosporins and aztreonam in the treatment of serious infections caused by these pathogens. Though presence of ESBLs amongst *E. coli* have been reported from Iran (Aminzadeh et al., 2008; Bazzaz et al., 2009), there is no information on their molecular types. The present study was carried out to determine the prevalence of mainly TEM and SHV genes responsible for ESBL production amongst the ESBL positive *E. coli* species isolated from the patients admitted in Shahid Beheshti Hospital in Kashan, Iran.

MATERIALS AND METHODS

A total of 150 clinical isolates of *E. coli* received in the clinical bacteriology laboratory, Shahid Beheshti Hospital in Kashan, from August 2009 to July 2010 were included in this study. The isolates were identified by their cultural characteristics and reactions to standard biochemical tests (Bueris et al., 2007).

Isolates were tested by the double disk diffusion method on Mueller Hinton agar (Becton Dickinson Microbiology System, UK). ESBL detection was carried out following the CLSI (former NCCLS) (Figure 3) recommended method for screening and confirmation using cefotaxime and ceftazidime as substrates (Clinical Laboratory Standards Institute, 2006). For deoxyribonucleic acid (DNA) extraction, a boiling method for release of DNA from cells was used (Aranda et al., 2004). DNA was extracted from bacteria by re-suspending one bacterial colony in 100 μ l of deionized water, boiling the suspension for 10 min and centrifuging it at 14,000 \times g for 5 min. The supernatant was then used as the DNA template for PCR.

The optimized protocol was carried out with a 25 μ l mixture containing 13.5 μ l deionized water, 2.5 μ l 10X PCR buffer, 1 mM MgCl₂, 1 mM concentration of each deoxynucleoside triphosphate, 0.5 U of Taq DNA polymerase (Fermentas, Germany) 5 μ l of the DNA template, and 1 μ l of primers mix 1 or 2 containing 10 ng primer/ μ l. The primer sequences and cycling conditions used in the PCR (Thermal cycler, Bioneer SouthKorea) analysis are listed in Table 1. The PCR product bands were separated by electrophoresis through 1.2% agarose gel in 1 \times TBE buffer (Figures 1 and 2). DNA fragments were visualized by ethidium bromide staining and photographed under ultraviolet light illumination (Khorshidi et al., 2011; Motallebi et al., 2011). All data were entered

into SPSS software and comparative statistics was evaluated.

RESULTS

In this study, 150 strains of *E. coli* were obtained from clinical specimens. These specimens contained 70 strains of ESBL phenotype (23 males and 47 females) (Table 2). 63 of the 70 strains were isolated from urine specimens (90%), one specimen from tracheal tube (5.1%), 2 specimens from blood (3%), 3 specimens from wounds (4%) and one specimen from CSF (5.1%).

The rates of blaTEM-1 and blaSHV-1 in the isolates were 44(63%) and 5(7%), respectively. In 9 (13%) of the isolates, both blaTEM-1 and blaSHV-1 were detected and 12 samples were free of these genes.

DISCUSSION

Enterobacteriaceae family members are major causes of nosocomial and community acquired infections and some of them as members of the normal flora can cause opportunistic infections. Therefore, adopting a new strategy to treat, diagnose and control the spread of these strains is essential. Antibiotic treatments of these infections were readily available in recent years with the penicillin antibiotics family, but today these treatments are difficult and costly because of acquired ESBLs resistance.

Various studies showed that the percentage of ESBL-producing *E. coli* strains in India (2004), Canada (2003), United States (2003), Spain (2005), Lebanon (2005), Korea (2004), Germany (2005), Palestine (2005), Bangladesh (2004), Turkey (2004) and France (2006) were 27, 63.8, 10 to 20, 51.8, 13.3, 9.2, 10.3, 22, 43.2, 17 and 4% respectively (Paterson and Bonomo, 2005). Duman et al. (2005), in a similar study, showed that 44.8% of the isolates of *Klebsiella pneumoniae* and 45.1% of all *E. coli* isolates produced ESBL.

A study in South Korea has reported that the prevalence of ESBL in *Klebsiella pneumoniae* and *E. coli* is 22.8 and 7.5%, respectively (Hawkey, 2008). Antibiotic resistance is increasing in developing countries than in developed countries (Yoshikawa et al., 1996). In a study by Kalantar et al. (2007), from 138 *E. coli* isolates, 68.1% ESBLs were produced by phenotypic method. Incidence rates of bla-TEM, bla-SHV and blaCTX-M among ESBL

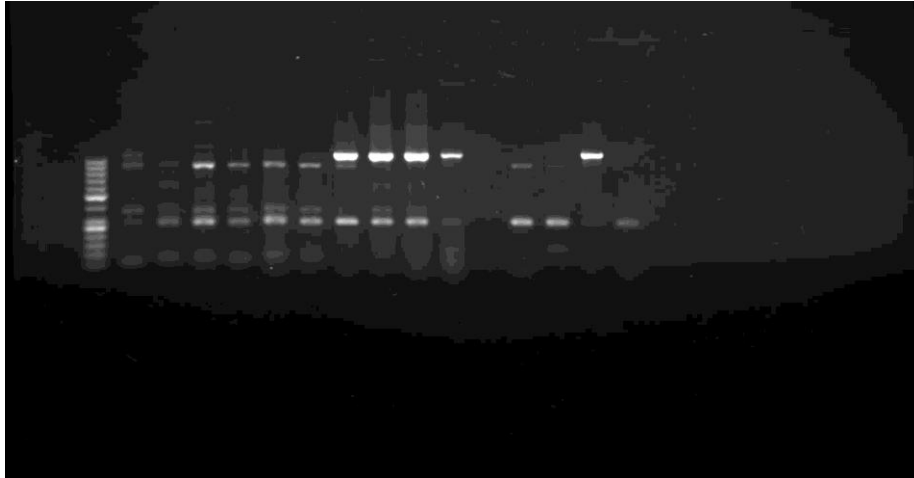


Figure 1. Agarose gel electrophoresis of products from PCR associated gene TEM-1 (Length 1080bp) *E. coli*. Lane 1, DNA molecular size markers (100-bp ladder); lane 8, 9, 10, 11, 15 positive sample (TEM-1).

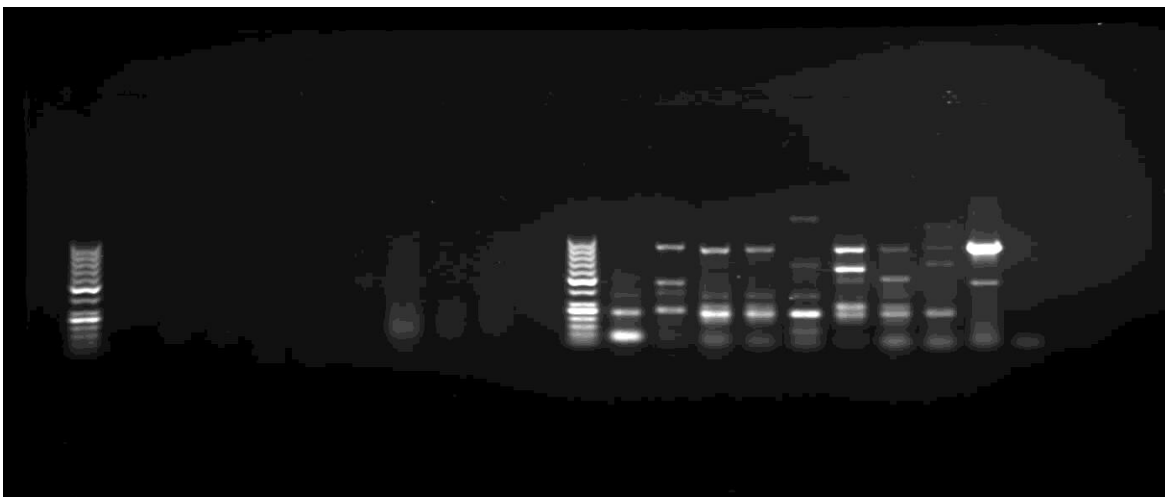


Figure 2. Agarose gel electrophoresis of products from PCR associated gene SHV-1 (Length 865bp) *E. coli*. Lane 1, DNA molecular size markers (100-bp ladder); lane 3, 4, 5, 7, 8, 9, 10 positive sample (SHV-1).

producing isolates were 63.8, 51 and 23.4%, respectively (Fereshteh et al., 2007). Our study showed that the rate of ESBL-producing strains among *E. coli* isolates was 46.6%. This value was more than our primary forecast, but it was less than that of Hosseini-Mazinani's study in Iran. Our study showed that 74.3% of people infected with the bacteria producing ESBL were over 40 years old (Hosseini-Mazinani et al., 2007). Pitout and Laupland, (2008) study found that patients of over 65 years old are more susceptible to infection caused by these organisms.

Also in our study of 70 cases with a phenotype of ESBL, 43 patients (60%) had an underlying disease that almost all of these people were aged over 50 years. This means that the underlying disease and age have

increased risk of infections caused by these organisms. Shahcheraghi et al. (2007) study in Tehran showed 24% of *E. coli* strains samples had genes bla TEM; 6 percent, bla SHV and 3% had both genes. The results of our study are in agreement with some studies in Iran and this indicates that most genes that cause TEM resistance to broad-spectrum antibiotics in *E. coli* are TEM1 (Shahcheraghi et al., 2007).

Conclusion

The prevalence of ESBL is very low in some countries where accurate nosocomial infection controls have been



Figure 3. A positive sample using double-disk diffusion antibioticgram.

Table 2. Frequency of ESBL-producing phenotypes from hospitalized patients in Kashan in 2009-2010 based on age (years).

Age (year)	Frequency (number)	Percent (%)
1-40	18	25.7
40-85	52	74.3
Total	70	100

applied and where there is proper control of antibiotic consumption. Therefore, to control these strains, it is necessary to supervise hospitals and health centres strictly. Given the high levels of resistance, accurate antibiotic tests before prescribing antibiotics and avoiding indiscriminate use of antibiotics are essential.

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