Molecular Epidemiology of ESBLs Genes and Multi-Drug Resistance in Diarrheagenic Escherichia Coli Strains Isolated from Adults in Iran

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Abstract

Resistance to oxyimino cephalosporins antibiotics in Enterobacteriaceae is primarily done by the extended spectrum β-lactamases (ESBLs). Clear identification of risk factors for ESBLs-producing infections is necessary. Therefore, efficient strategies can be developed to decrease outbreak of these infections. The aim of this study was to determine the antibacterial susceptibility and ESBLs pattern of diarrheagenic Escherichia coli (E. coli) strains isolated from adult patients. In the present study, diarrheagenic E. coli strains were isolated from 54 patients from the University of Medical Sciences hospitals in Shiraz. Antimicrobial susceptibility testing was done by disk diffusion method by CLSI criteria. The presence of blaTEM, blashiV, and blaCTX-M genes was investigated by PCR using designated primers. The prevalence of ESBLs-producer E. coli strains was 12.96%. Antimicrobial resistance testing showed a high resistance to cefexime, trimethoprim-sulfamethoxazole, ampicillin and penicillin. Overall, β-lactamase genes were identified in 52 (96.30%) isolates which were identified as 45 (83.33%) blaTEM, 17 (31.48%) blashiV and 11 (20.37%) blaCTX-M ESBLs-producer E. coli is very prevalent in diarrheagenic strains isolated from adult patients. Also, this study clearly showed that the blaTEM gene for ESBLs-producer E. coli was widespread in Iran.

Keywords: Antibiotic resistance; E. coli; ESBLs; MDR.

Introduction

Diarrhea is one of the world’s widespread health problems, with more than two million deaths in each year. Diarrheal disease is caused by a range of enteric pathogens such as viruses, bacteria, and parasites. Diarrheagenic Escherichia coli (DEC) and Shigella spp. are the most popular bacteria causing diarrhea (1). Emergence and dispersion of antibiotic resistance is well documented in bacterial isolates worldwide, particularly in developing countries (2, 3). Antibiotic resistance prevalence in E. coli is an effective marker for antibiotic resistance in each community (2, 4). E. coli is famous to be efficiently capable of accepting and transferring genetic materials and, under stress, readily transfers those genetic materials to enteric pathogens including Salmonella,
Yersinia, Vibrio, and Shigella species. Therefore, it is considered an important reservoir of transferable antibiotic resistance (2-4). There is a global interest regarding advent and the rise of resistance to generally used antibiotics in bacteria (5). Irregular usage of antibiotic is possibly more important factor promoting the emergence, selection, and distribution of antibiotic-resistant bacteria in human and veterinary medicine (6). This global usage of antibiotics could be connected with the selection of antibacterial resistance mechanisms in both nonpathogenic and pathogenic strains of E. coli. β-lactams are one of the most widely used antibiotics in both human and veterinary medicine (4). β-lactam resistance in Enterobacteriaceae is primarily conducted by β-lactamases. However a range of β-lactamases have been characterized, typically TEM, CTX-M, and SHV enzymes are those prevalently observed among Enterobacteriaceae and have been increasingly found throughout the world (7, 8). Mutations in the genes encoding these enzymes can increase the spectrum of the activity of enzyme to include penicillins, the extended-spectrum cephalosporins, and aztreonam. These enzymes are named extended-spectrum β-lactamases (ESBLs) (8). Antimicrobials resistant bacteria is a global problem (9). Routine monitoring of antibiotic resistance is important in order to prepare data for antibiotic therapy and resistance control (2). Also, understanding the molecular foundations of resistance can play an important role in the progress of new strategies to fight against this phenomenon (9). It is mandatory to clearly identifying the hazardous factors for infections owing to ESBLs-producing bacteria, therefore efficient strategies to decrease spread of these infection agents can be developed. Various studies have been done in an attempt to identify hazardous factors for infections owing to ESBLs-producer bacteria, but the results have been largely different. However these differences could be due in part to real disparity in the epidemiology of various outbreaks (10).

The aim of the present study was to determine the types of β-lactamases and antimicrobial susceptibility pattern of DEC strains recovered from diarrheal specimens of adult patients with diarrhea in the south of Iran.

Experimental

Samples collection
During the period from March, 2010 to December 2010, a total of 54 DEC were collected from patients with diarrhea admitted to all of the university hospitals in Shiraz. Written informed consent was obtained from all patients. All steps of this study were approved by the Ethical Committee of Islamic Azad University. Initial isolation of samples was performed on MacConkey, EMB and VRBA and confirmed by using standard biochemical tests, including indole, TSI, citrate, urea, MR, VP, LD, OD and SIM tests (Merck, Germany).

Phenotypic detection of ESBLs production
A modified version of the Jarlier double-disk synergy (DDS) method (11) for detecting calvulanic acid (CLA) synergy was used. Ceftazidime (30 µg) and cefotaxime (5 µg) disks (Oxoid), were placed around an amoxicillin (20 µg)-clavulanic acid (10 µg) disk at a distance of 25 to 30 mm from center to center. A clearly visible extension of the edge of the inhibition zone of any disk towards the amoxicillin-clavulanic acid disk was interpreted as positive for CLA synergy (11).

Antimicrobial susceptibility testing
Isolates were subjected to standard disc diffusion testing in accordance with the recommendation of the Clinical Laboratory Standard Institute (12). Isolates were tested for resistance to the following antibiotics: cefotaxime (30 μg), ceftriaxone (30 μg), cefexime (5 μg), ampicillin (10 μg), penicillin (10 U), imipenem (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), chloramphenicol (30 μg), tetracycline (30 μg), sulfamethoxazole–trimethoprim (1.25 and 23.75 μg), gentamicin (10 μg), amikacin (30 μg) and nitrofurantoin (300 μg) (Mast Diagnostics, Merseyside, UK). Quality control was performed as recommended using the E. coli strain ATCC 25922.

DNA extraction
E. coli isolates were grown overnight in 5 ml of Luria Bertani (LB) broth (Merck) at 37°C. One milliliter of cell suspension for each isolate was transferred to 1.5-ml tubes and centrifuged at 13000 rpm for 5 min. The supernatants were
removed and the cell pellets resuspended in 200 µl of sterile water by spinning. The suspensions were boiled for 15 min to lyse the cells and centrifuged as before. 150 µl of each supernatant containing DNA was removed for testing.

**PCR of β-lactamase-encoding genes**

The detection of β-lactamase-encoding genes was carried out using multiplex polymerase chain reaction (PCR) with primers that correspond to conserved regions of $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$-type genes (Table 1). PCR amplification was performed in a reaction mixture of 25 µL, which contained 2 µL template DNA, 1.5 mM MgCl$_2$, 0.2 mM deoxynucleoside triphosphates (dNTPs) mixture, 0.2 mM of each primer, and 1 U of Taq DNA polymerase (CinaGen, Co., Tehran, Iran). Initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min; and, a final extension at 72°C for 5 min were used as thermal-cycling conditions.

**E. coli ATCC 35218** carrying $\text{bla}_{\text{TEM}}$ and **Klebsiella pneumoniae ATCC 700603** harboring $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$ were used as a positive control of amplification and **E. coli ATCC 25922** was used as a negative control. The amplicons were visualized after electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

**Results**

**Description of sample population**

The prevalence of infected females, (53.70%) was higher than that of infected males, (46.30%). The age of patients were varied from 19 to 65 years of age. Out of the 54 **E. coli** collected, 17 (31.48%) were identified in the spring, 20 (37.04%) were in the summer, and 17 (31.48%) were in the autumn. The clinical symptoms included nausea 38 (70.37%), fever 29 (53.70%) and dysentery 7 (12.96%) (Table 2).

**Antimicrobial resistance E. coli**

The prevalence of resistance to each antimicrobial agent was: cefotaxime 16.67%, ceftriaxone 16.67%, cefexime 30.56%, imipenem 5.56%, ampicillin 36.11%, penicillin 100%, ciprofloxacin 8.33%, levofloxacin 5.56%, chloramphenicol 13.89%, tetracycline 41.67%, trimethoprim-sulfamethoxazole 41.67%, gentamycin 8.33%, amikacin 5.56% and nitrofurantoin 5.56%.

**Phenotypic results for ESBL’s**

The overall incidence of ESBL producing isolates was 12.96% (7/54) of **E. coli** during the study period. All isolates that tested positive for ESBLs were also resistant to more than 4 antibiotics (multi-drug resistance).

**Molecular characterization of ESBL genes**

Out of 54 isolates that were subjected to PCR experiments, 52 (96.30%) of them harbored ESBLs genes. $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$ genes were detected in 45 (83.33%), 17 (31.48%) and 11 (20.37%) of the samples, respectively. Seventeen out of 54 isolates (31.48%) carried several $\text{bla}$ genes (more than one gene) while 35/54 (64.82%) of samples harbored a single $\text{bla}$ gene (Table 3).

**Discussion**

Diarrheal disease owing to the different diarrheagenic **E. coli** strains is a health problem around the world, especially in developing countries. Also, it has contributed exceedingly to morbidity, mortality and increased health costs (14, 15, 16). Diarrheal diseases are rank as the fourth prevalent cause of mortality, and it has been considered that infectious diseases cause 9.2 million deaths in the developing countries.
Antibiotic resistant diarrheagenic *E. coli* strains are usually associated with β-lactam antibiotics. In order to efficient use of antibiotics in clinical management and treatment, continuous monitoring of antimicrobial resistance is absolutely necessary (14, 17, 18, 19). Hospital transfer and antibiotic usage, particularly oxyiminocephalosporins, are well established risk factors for the distribution of ESBLs producing bacteria (18). This study prepares information regarding the problem of antibiotic resistance in diarrheagenic *E. coli* strains isolated from patients enrolled in clinics, hospitals and outpatient facility. Results showed that these *E. coli* strains have elevated rates of resistance to the currently prescribed antibiotics. Resistance against penicillins and fulate inhibitors was very high. Ampicillin resistance among *E. coli* strains was 36.11%, which is lower than the other study in Iran (14). The ampicillin resistance among diarrheagenic *E. coli* is probably due to continuous use of it for many years (20, 21). The imipenem resistance was found to be 5.56%. Imipenem is a carbapenem antibiotic, and is highly active against ESBLs producing *Enterobacteraceae*. This drug is highly resistant to beta-lactamase and has an unusual property. It causes a post antibiotic effect on Gram-negative bacteria (22). Gentamicin resistance was found to be 8.33%, which is low as compared to study reported by Aslani *et al.* (14). However, in our study, we found a 5.56% resistance to amikacin and found it be a more effective aminoglycoside against diarrheagenic *E. coli*. The resistance rate was low against aminoglycosides *i.e.*, gentamicin and amikacin, but it was equal to carbapenem, nitrofurantoines and fluoroquinolones. Resistance to ciprofloxacin was 8.33% in our study, which is in agreement with another report from Iran (14). This resistance may be owing to the use of fluoroquinolones as the drug of choice in urinary tract infections (UTI) (20). The resistance rates reported in this study is in agreement with some reports from Vietnam (1), Nigeria (3), Iran (14) and Brazil (22) but are lower than Spain (7), USA (10), Thailand (18) and Pakistan (20). Many factors may have been involved with increased rates of antibiotic resistance including: mishandle of antibiotics by health care professionals or non-skilled practitioners, misuse of antibiotics

| Table 2. Description of different criteria of patients in order to gender (n = 54). |
|-----------------------------------------------|-----------------|-----------------|
| Male (%)                                       | Female (%)      |
| 18-23                                          | 8 (14.81)       | 5 (9.26)        |
| 24-35                                          | 8 (14.81)       | 13 (24.07)      |
| 36-47                                          | 3 (5.56)        | 7 (12.96)       |
| 48-60                                          | 5 (9.26)        | 1 (1.85)        |
| >60                                            | 1 (1.85)        | 3 (5.56)        |
| Spring                                         | 6 (11.11)       | 11 (20.37)      |
| Summer                                         | 9 (16.67)       | 11 (20.37)      |
| Fall                                           | 10 (18.52)      | 7 (12.96)       |
| Nausea                                         | 17 (31.48)      | 21 (38.89)      |
| Fever                                          | 13 (24.07)      | 16 (29.63)      |
| Dysentery                                      | 5 (9.26)        | 2 (3.70)        |
| TEM                                            | 21 (38.89)      | 24 (44.44)      |
| SHV                                            | 10 (18.52)      | 7 (12.96)       |
| CTX-M                                          | 3 (5.56)        | 8 (14.81)       |

| Table 3. Distribution of ESBLs genotype of 54 diarrheagenic *E. coli*. |
|-----------------------------|------------------------|------------------------|------------------------|
| Single Gene | Multiple Gene | None |
| TEM | SHV | CTX-M | TEM+SHV | TEM+CTX-M | SHV+CTX-M | TEM+SHV+CTX-M |
| 30 (55.56) | 3 (5.56) | 2 (3.70) | 8 (14.81) | 3 (5.56) | 2 (3.70) | 4 (7.41) | 2 (3.70) |
by the general public (antibiotics can be used in some regions without a doctor’s prescription from pharmacy), and inadequate surveillance due to a lack of information arising from routine antimicrobial susceptibility testing, such as reports from other developing countries (20, 21, 22). Controlling the emergence and spread of ESBLs organisms involves a combination of controlling antibiotic use and strict adherence to hospital infection control measures. Restriction of one class of antibiotics can lead to increased use of another class with an accompanying increase in resistance rates. Attempts have been made to decrease the prevalence of ESBLs producing organisms by substituting earlier cephalosporins with a fourth-generation cephalosporin or beta-lactam/beta-lactamase inhibitor combinations (20). Cefotaxime and ceftiraxone resistance in E. coli strains described in this paper was largely associated with TEM beta-lactamase genes, with only one isolate negative for TEM beta-lactamase gene, but positive for both SHV and CTX-M beta-lactamase genes. Although CTX-M types of ESBLs have been known for their rapid spread in many parts of the world (18, 23-27), it was remarkable that in this study, blaTEM is more prevalent than other types of ESBLs gene in diarrheagenic E. coli strain. This finding is in agreement with other studies in center (14, 28, 29) and south (30-33) of Iran that showed blaTEM is more prevalent in E. coli. In conclusion, E. coli involved in diarrhea isolated from patients enrolled in hospitals and clinics showed resistance to many antimicrobial agents, especially penicillin, ampicillin, trimethoprim-sulfamethoxazole, and cefixime, resulting in a very high percentage of resistance isolates. Also, the high number of ESBLs producing isolates gives rise to concern. This study clearly showed that the TEM beta-lactamase gene, for ESBLs producing E. coli, was highly endemic in Iran. Regular monitoring of resistance to antimicrobial drugs and ESBLs would seem to be necessary to improve our guidelines for empirical antibiotic therapy.

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References

(1) Hien BTT, Scheutz F, Cam PD, Serichantalergs O, Huong TT, Thu TM and Dalsgaard A. Diarrheagenic Escherichia coli and Shigella strains isolated from children in a hospital case-control study in Hanoi, Vietnam. J. Clin. Microbiol. (2008) 46: 996-1004.
(2) Okeke IN, Fayinka ST and Lamikanra A. Antibiotic resistance in Escherichia coli from Nigerian students, 1986-1998. Emerg. Infec. Dis. (2000) 6: 393-396.
(3) Aibinu IE, Peters RF, Amisu KO, Adesida SA, Ojo MO and Odugbemi T. Multidrug resistance in E. coli 0157 strains and the public health implication. J. Am. Sci. (2007) 3: 22-33.
(4) Brinas L, Zaragaza M, Sa’enz Y, Ruiz-Larrea F and Torres C. beta-Lactamases in ampicillin-resistant Escherichia coli isolates from foods, humans, and healthy animals. Antimicrob. Agents Chemother. (2002) 46: 3156-3163.
(5) Lietzau S, Raum E, von Baum H, Marre R and Brenner H. Clustering of antibiotic resistance of E. coli in couples: suggestion for a major role of conjugal transmission. BMC Infect. Dis. (2006) 6: 119.
(6) Miles TD, McLaughlin W and Brown PD. Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. BMC Vet. Res. (2006) 2:7.
(7) Oteo J, Navarro C, Cerenceno E, Delgado-Iribarren A, Wilhelmi I, Orden B, Garcia C, Migueláñez S, Pérez-Vázquez M, García-Cobos S, Aracil B, Bautista V and Campos J. Spread of Escherichia coli strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J. Clin. Microbiol. (2006) 44: 2359-2366.
(8) Steward CD, Rasheed JK, Hubert SK, Biddle JW, Raney PM, Anderson GJ, Williams PP, Brittain KL, Oliver A, McGowan JE Jr. and Tenover FC. Characterization of clinical isolates of Klebsiella pneumoniae from 19 laboratories using the national committee for clinical laboratory standards extended-spectrum beta-lactamase detection methods. J. Clin. Microbiol. (2001) 39: 2864-2872.
(9) Ahmed MO, Clegg PD, Williams NJ, Baptiste KE and Bennett M. Antimicrobial resistance in equine faecal Escherichia coli isolates from North West England. Ann. Clin. Microbiol. Antimicrob. (2010) 9: 12.
(10) Lautenbach E, Patel JB, Bilker WB, Edelstein PH and Fishman NO. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. Clin. Infect. Dis. (2001) 32: 1162-1171.
(11) Jarlier V, Nicolas MH, Fournier G and Philippou A. Extended-broad spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility.
patterns. Rev. Infect. Dis. (1988) 10: 867-78.

(12) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Twenty-first informational supplement. CLSI document M100-S21, The Institute, Wayne (2011).

(13) Sturenburg E, Kuhn A, Mack D and Lauß R. A novel extended-spectrum β-lactamase CTX-M-23 with a P167T substitution in the active-site omega loop associated with ceftazidime resistance. J. Antimicrob. Chemother. (2004) 54: 406-409.

(14) Aslani MM, Salmanzadeh-Ahrabi S, Alikhani YM, Jafari F, Zali RM and Mani M. Molecular detection and antimicrobial resistance of diarrheagenic Escherichia coli strains isolated from diarrheal cases. Saudi Med. J. (2008) 29: 388-392.

(15) Akinjogunla OJ, Eghafona NO and Ekoi OH. Diarrheagenic Escherichia coli (DEC): prevalence among in and ambulatory patients and susceptibility to antimicrobial chemotherapeutic agents. J. Biotech. Res. (2009) 1: 34-38.

(16) Montaz H, Karimian A, Madani M, Safarpooor Dehkordi F, Ranbar R, Sarshar M, Soud N. Uropathogenic Escherichia coli in Iran: Serogroup distributions, virulence factors and antimicrobial resistance properties. Ann. Clin. Microbiol. Antimicrob. (2013) 12: 8.

(17) Montaz H, Dehkordi FS, Hosseini MJ, Sarshar M, Heidari M. Serogroups, virulence genes and antibiotic resistance in Shiga toxin-producing Escherichia coli isolated from diarrheic and non-diarrheic pediatric patients in Iran. Gut Pathog. (2013) 5(1): 39.

(18) Kiratsin P, Apisarnthanarak A, Laestripa C and Saifon P. Molecular characterization and epidemiology of extended-spectrum-β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates causing healthcare-associated infection in Thailand, where the CTX-M family is endemic. J. Antimicrob. Chemother. (2008) 52: 2818-2824.

(19) Salehifar E, Nashei M, Elsami G, Sahraei S, Alizadeh Navaee R. Determination of antibiotics consumption in buali-sina pediatric hospital, sari 2010-2011. Iran. J. Pharm. Res. (2014) 13: 995-1001.

(20) Hassan SA, Jamal SA and Kamal M. Occurrence of multidrug resistant and ESBL producing E. coli causing urinary tract infections. J. Basic Appl. Sci. (2011) 7: 39-43.

(21) Sarrafzadeh F, Soheivardi SM. Evaluation of Bacteruria and Antimicrobial Susceptibility among Hospitalized Patients With and Without Catheter in Kerman Province-Iran in 2011. Iran. J. Pharm. Res. (2013) 12: 211-6.

(22) Santo E, Salvador MM and Marin JM. Multidrug-resistant urinary tract isolates of Escherichia coli from Ribeirão Preto, São Paulo, Brazil. Braz. J. Infect. Dis. (2007) 11: 575-578.

(23) Tnfteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, Sundsfjord A and Norwegian ESBL Study Group. Effects of phenotype and genotype on methods for detection of extended-spectrum-β- lactamase-producing clinical isolates of Escherichia coli and Klebsiella pneumoniae in Norway. J. Clin. Microbiol. (2007) 45: 199-205.

(24) Farber J, Moder KA, Layer F, Tamler I, König W and König B. Extended-spectrum beta-lactamase detection with different panels for automated susceptibility testing and with a chromogenic medium. J. Clin. Microbiol. (2008) 46: 3721-3727.

(25) Kim MH, Lee HJ, Park KS and Suh JT. Molecular characteristics of extended spectrum β-lactamases in Escherichia coli and Klebsiella pneumoniae and the prevalence of qnr in extended spectrum β-lactamase isolates in a tertiary care hospital in Korea. Yonsei Med. J. (2010) 51: 768-774.

(26) Zhaneg GG, DeCorby M, Adam H, Mulvey MR, McCracken M, Lagoç-Wiens P, Nichol KA, Wierzbowski A, Baudry PJ, Tailor F, Karlowsky JA, Walkty A, Schweizer F, Johnson J, Canadian Antimicrobial Resistance Alliance and Hoban DJ. Prevalence of antimicrobial-resistant pathogens in Canadian hospitals: results of the Canadian Ward Surveillance Study (CANTWORTH 2008). Antimicrob. Agents Chemother. (2010) 54: 4684-4693.

(27) Jones CH, Tuckman M, Keeney D, Ruzin A and Bradford PA. Characterization and sequence analysis of extended-spectrum-β-lactamase-encoding genes from Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates collected during tigecycline phase 3 clinical trials. Antimicrob. Agents Chemother. (2009) 53: 465-475.

(28) Mirsaleheian A, Akbari-Nakhjavani F, Peymani A, Kazemi B, Jabal-Ameli F and Miraftab SM. Prevalence of extended spectrum β-lactamase-producing Enterobacteriaceae by phenotypic and genotypic methods in intensive care units in Tehran, Iran. Daru J. Pharm. Sci. (2008) 16: 169-173.

(29) Hosseini-Mazinani SM, Effekhar F, Milani M and Ghandiidi S. Characterization of β-lactamases from urinary isolates of Escherichia coli in Tehran. Iran. J. Biotech. (2007) 11: 95-99.

(30) Kargar M, Kargar M, Jahromi MZ, Najafi A, Ghorbani-Dalini S. Molecular detection of ESBLs production and antibiotic resistance patterns in Gram negative bacilli isolated from urinary tract infections. Indian J. Pathol. Microbiol. (2014) 57: 244-248.

(31) Ghorbani-Dalini S, Kargar M, Doosti A, Abbasi P, Sarshar M. Multidrug-resistant (MDR) ESBLs producing diarrheagenic E. coli strains isolated from pediatric patients. Iran. J. Infec. Dis. Trop. Med. (2012) 17: 13-19.

(32) Kargar M, Gholami M, Doosti A, Najafi A and Aein V. Frequency of extended spectrum β- lactamase producing Escherichia coli in hospitalized and out patient children. Armaghane Danesh (2014) 3: 212-222.

(33) Mortazavi R, Khosravani A and Naghavi NS. Molecular analysis of gene frequencies of TEM, CTX-M and SHV in beta-lactam antibiotic-resistant strains of E. coli isolated from urinary tract infections in Yasuj hospitals. Armaghane Danesh (2014) 3: 233-241.

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