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Antibiotic resistance pattern and assessment of Temorina gene in clinical strains of extended-spectrum beta-lactamase enzyme producing *Escherichia coli* isolated from patients, Babol City, Mazandaran Province

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ABSTRACT

Introduction: *Escherichia coli* (*E. coli*) bacteria are a common cause of various clinical infections. Resistance of this bacteria to several common antibiotics due to production of extended spectrum beta-lactamase (ESBL) enzyme has caused therapeutic problems. The aim of this study was to determine the resistance pattern to beta-lactam antibiotics and also to assess the Temorina (TEM) gen in the *E. coli* strains isolated from the patients in Babol, Iran.

Methods: This cross-sectional study was conducted in 2014 at Babol County, Iran. The *E. coli* strains were isolated and identified by standard laboratory tests. The sensitivity test to beta-lactam antibiotics was performed by combined disk method. The TEM gene was identified in the resistant strains by the polymerase chain reaction (PCR) method. The data were analyzed by SPSS 20 and by using T-test and Chi-squared tests.

Results: Of the 10,341 clinical samples, 525 *E. coli* isolated of which 200 (38%) were ESBL-producing strains. Piperacillin-tazobactam, amikacin, ampicillin-sulbactam and ampicillin (98%, 90.33%, 86.4% and 76.60%, respectively) had the most inhibition effect on the strains. Highest antibiotic resistance was observed for ceftriaxone (43.80%) and ciprofloxacin (38.74%). PCR showed that 80% (n=160) of the resistant strains had the TEM gene. There was a significant correlation between TEM gene and the production of ESBL ($P < 0.05$).

Conclusion: Resistance to antibiotics was observed in this study. Resistant and ESBL-producing strains of *E. coli* had TEM gene. The clinicians should be aware of antibiotic resistant pattern to choose effective medicines for treatment of these infections.

Introduction

Escherichia coli (*E. coli*) is the cause of various illnesses including urinary infections, sepsis and nosocomial infections (1-3). Some bacterial species produce antibiotic resistant genes by changed plasmids that produce extended spectrum beta-lactamase (ESBL) enzymes. Beta-lactamase is an enzyme that makes the bacteria resistant to several antibiotics (4). Antibiotic resistances are different in various places of a country or the world due to the genetic changes of the strains, differences in antibiotic use, and differences in access to the antibiotics (5). Emergence of the bacteria that can produce ESBL is a critique in treatment of bacterial infections (6). Studies have shown that the patients who are affected by infections by ESBL-producing bacteria, experience a higher rate of mortality compared with those strains that do not produce ESBL (7, 8). Production of beta-lactamases is the main cause of resistance to the beta-lactam antibiotics. So because of arbitrary use of antibiotics, the prevalence of infectious epidemics due to ESBL is significantly increasing worldwide (9). The TEM gene usually is seen in *E. coli* and other gram-negative bacteria (10). Thus the widespread use

of extended spectrum cephalosporin antibiotics leads to spread of ESBLs (9). The TEM gene is one of the genes related to ESBL and more than 150 type of ESBL are reported from various countries which are produced by the Enterobacteriaceaes (11, 12). The prevalence of TEM gene is reported to be 30% in India and 72% in Turkey (12, 13). Its prevalence in strains of *E. coli* in Iran also reported to be 84% (14). Considering the above mentioned statements, investigation of antibiotic resistance in gram-negative bacteria is important for correct use of antibiotics in treatment of the infections. Utilization of antibiotic resistance data results in reduction of problems related to antibiotic resistance, prescription of proper antibiotics, and control of bacterial contamination in hospital wards. The aim of this study was to determine the resistance pattern to beta-lactam antibiotics and also to assess the Temorina (TEM) gen in the *E. coli* strains isolated from the patients in Babol, Iran.

Methods

This was a cross-sectional study performed at the Shahid Yahyanejad Hospital of Babol, Iran, July to December 2014. Study populations were the 10,341 patients admitted at the intensive care unit (ICU), coronary care unit (CCU), infection, internal, skin, emergency, and heart and surgery wards of the hospital. The study protocol was reviewed and approved by the institutional review board at Research Deputy of Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran. All patients (census sampling method) in Shahid Yahyanejad Hospital were potentially eligible to include in the study. Inclusion criteria were all patients who had infection according to laboratory results and medical confirmation. Samples of urine, respiratory secretions, ulcers, blood, body fluids (ascites, pleural and spinal fluids) of the patients that were tested. The samples were cultured on blood agar, MacConkey and chocolate agar (Merck, Germany) environments and then incubated. To identify the bacteria, the direct observation method by gram staining and the biochemical tests of glucose and lactose fermentation test, indole test, methyl-red reaction and voges proskauer, Simmons' citrate test, and urease test were applied (13). To isolate the strains that produce the ESBL, the combined disk method was applied by using the 0.5 McFarland microbial suspension, Muller Hinton Agar environment, and disks of Cefotaxime (30 microgram), combination of Cefotaxime (30 microgram)-Clavulanic acid (10 microgram), Ceftazidime (30 microgram), combination of Ceftazidime (30 microgram)-Clavulanic acid (10 microgram), Cefepime (30 microgram), and the combination of Cefepime (30 microgram)-Clavulanic acid (10 microgram). After incubation for 18-24 hours in 35 °C, the halo sizes on the disks were measured and the halo size of the disks which contained Clavulanic acid were compared to the ones that did not contain. According to the Clinical and Laboratory Standards Institute (CLSI), if the halo size of the combined disks were 5≤ millimeter bigger than the simple disks, the strain was considered as the ESBL producing. The disk release method also was applied according to the CLSI by using antibiotics of Ceftriaxone (30 microgram), Ciprofloxacin (5 microgram), Piperacillin-Tazobactam (110 microgram), Amikacin (10 microgram), Ampicillin-Sulbactam (20 microgram), and Imipenem (10 microgram) (MAST, UK) (12, 15). After cultivation on Luria-Bertani Broth medium (Merck, Germany), the DNA of all ESBL producing strains were extracted according to the instruction of Takapouzist Company. The TEM gene was traced by specific primers and thermal scheme in Polymerase Chain Reaction (PCR) according to Table 1 and 2 (16). The data collection tool was the standard questionnaire of the Health Reference Laboratory. Since the required data were gathered from the hospital information system (HIS) and since the data were anonymous, there was no need for consent of the patients. The data were analyzed by the SPSS software version 20 and using the T-test for quantitative and Chi square test for qualitative variables. Significance level was considered as $P < 0.05$.

Results

Overall 10,341 people were referred to the study hospital during the study period. The samples included 7350 urine samples, 2667 blood samples, 74 ulcer samples, 65 samples of respiratory secretions, 13 samples from throat, 26 samples of joins, 45 samples of spinal fluid, 35 samples of ascites, 13 samples of pleural fluid, 50 samples from shunts, and 3 samples from the abscess.

Table 1. The used primers at the PCR

Primer name	Nucleotide sequence	Amplicon size
TEM-F	5 '–ATAAAATTCTTGAAGACGAAA-3'	1051 bp
TEM-R	5 ' – GACAGTTACCAATGCTTAATCA-3'	-

Of the studied patients, 65% (6722) were female and the rest were male. Moreover, 51.6% of them were from inpatient wards and 48.4% were outpatients. Of the abovementioned samples, 525 strains of *E. coli* were isolated of which 42% were from outpatients and 58% from inpatients. Majority of them (N=435) were isolated from the urine samples. A total of 200 strains (38%) were ESBL producing of which 28% were from outpatients and 72% from inpatients. Majority of the 200 ESBL producing strains (79%) were isolated from urine samples. The age range of the patients was 1-90 years. The ESBL producing strains of *E. coli* were isolated from urinary infection patients (34.65%), diabetic patients (25.6%), renal diseases patients (20%), heart diseases patients (18.4%) and digestive diseases (1.35%). Results of the antibiotic sensitivity test are presented at Table 3. As it is seen in this table, the antibiotics of piperacillin-tazobactam, amikacin, ampicillin-sulbactam, and imipenem had the highest inhibitory effect on ESBL producing strains of *E. coli*: 98%, 90.33%, 86.4%, and 76.6% respectively. The highest antibiotic resistance of the studied strains was towards the ceftriaxone (43.8%) and ciprofloxacin (38.74%). Results of the PCR showed that 160 (80%) of the ESBL producing strains had the TEM gene. There was a significant relationship between frequency of TEM gene, underlying diseases and inpatients with production of the ESBL ($P < 0.05$). Results of the possible correlation of the studied variables and the production of ESBL are provided in Table 4.

Discussion

Widespread use of antibiotics has lead in antibiotic resistance. One of the mechanisms that results in resistance in the bacteria is the production of ESBL. So the identification of these strains and drawing their antibiotic sensitivity pattern seems necessary for each country and even within the countries (11, 12). The study by Bajpai et al. in India showed that of the 71 clinical samples of *E. coli*, 40 were ESBL producing and the PCR showed that 38 of them (95%) had the TEM gene. This indicates the high prevalence of the resistant strains among the studied patients with suspected urinary tract infection which can be due to long stay in hospital and various and long term treatments with antibiotics (17). Study of Xu et al. in China assessed 276 strains of *E. coli* and found that 17.6% of them were ESBL producing and had the cefotaximase (CTX) gene (18). Rajivgandhi et al. in India reported that of the 100 isolated *E. coli* strains from urine specimens of patients with urinary tract infection, 84% were ESBL producing and 60 of them were resistant to Ciprofloxacin. Moreover, the TEM gene was identified among the resistant strains (19). Study of Haghi et al. in Tehran, Iran found that 33% of the *E. coli* strains were producer of the

Table 2. Thermal scheme of PCR for the TEM gene

Number of cycles	Time	Temperature (centigrade)	Stages
1	3 minutes	94	Initial denaturation
	30 seconds	94	Denaturation
35	30 seconds	55	Annealing
	30 seconds	72	Extending
1	5 minutes	72	Final Extending

Table 3. Antibiotic sensitivity and resistance pattern of *E. coli* strains isolated from the patients in Babol hospital, 2014

Antibiotic	Sensitive number (%)	Resistant number (%)	Intermediate number (%)
Ciprofloxacin	341 (61.95)	183 (34.85)	1 (0.19)
Amikacin	466 (88.76)	47 (8.95)	12 (2.28)
Ceftriaxone	274 (52.19)	249 (47.42)	2 (0.38)
Piperacillin-Tazobactam	492 (93.71)	30 (5.71)	3 (0.57)
Ampicillin-Sulbactam	436 (83.04)	89 (16.95)	-
Imipenem	385 (73.33)	128 (24.38)	12 (2.28)

Table 4. Statistical tests for correlation of the studied variables

Independent variable	Dependent variable	P- value
Production of ESBL	Frequency of TEM gene	<0.05
	Patient age	>0.05
	Patient gender	>0.05
	Underlying diseases	<0.05
	Inpatients	<0.05

ESBL which is similar to the findings of this study. But the frequency of the TEM gene was 46.96% which is lower than this study (20). Another study in Tehran, Iran by Eslami et al. found that 14% of the *E. coli* strains that were isolated from patient various samples, were ESBL producing and 100% of them had the TEM gene. The prevalence of ESBL is lower than the current study but the prevalence of the TEM gene is almost similar to this study (21). Considering the correlation between the prevalence of the ESBL producing strains and the antibiotic resistance, the high frequencies found in this study can be due to inappropriate use and overuse of antibiotics that disregards the correct antibiotic sensitivity pattern (21, 22). Molaabaszadeh et al. in Tabriz, Iran studied 5701 isolated *E. coli* strains of patients with urinary tract infection and reported the sensitivity to Meropenem was 90.95% which is different with the 24.38% for the Imipenem in this study. They also reported the resistance to Ampicillin as 93.95% which is much different to the findings of this study (16.95%) (22). This differences may be due to the differences in the antibiotic use pattern in different regions (6). According to the epidemiologic studies, in the recent years there has been an increased incidence and prevalence of the ESBLs (23). This difference can be due to variety in hospital length of stay, underlying diseases, antibiotic overuse, use of vascular and urinary catheters, history of surgery, and inappropriate and insufficient antimicrobial therapies (24, 25). ESBL producing isolates outbreak in health centers can occur and it can easily transmit between the hospital environment and patients. Therefore, epidemiological survey of bacteria strains, for detection of antibiotic resistant patterns of bacteria strains is necessary (25).

Conclusion

Findings of this study showed that a considerable proportion of clinical samples of isolated *E. coli* from patients were producing ESBL. Piperacillin-tazobactam, amikacin, ampicillin-sulbactam and ampicillin were more effective antibiotics. TEM gene presence was proved in majority of the isolated *E. coli* in this study. Consideration of the results of antibiotic sensitivity test can help choosing the proper antibiotic to start and continue the appropriate therapy in infection diseases.

Ethical disclosure

Since the required data were gathered from the hospital information system (HIS) and since the data were anonymous, there was no need for consent of the patients.

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Author contributions

F Z and E F supervised the study, participated in designing and conducting it. Z ED performed laboratory work and helped with the writing and editing of the manuscript. S R, K S, S A and P Sh collected the data and helped with the writing and editing of the manuscript.

Conflict of interest

The authors declare there is no conflict of interest.

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