The Association Between Genetic Variants in Extended Spectrum Beta-Lactamases and AmpC-producing Gram-Negative Bacilli and Antibiotic Resistance

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ABSTRACT

Background and Aim: A large group of genes associated with extended-spectrum beta-lactamases (ESBL) and AmpC expression is involved in inducing antibiotic resistance in various bacteria. Identifying these genes and assessing their harboring will be crucial in determining the pattern of antibiotic resistance. This study aimed to characterize genetic variants in extended-spectrum beta-lactamases, AmpC-producing gram-negative bacilli, and associated antibiotic resistance.

Materials and Methods: This cross-sectional research was conducted on clinical samples of patients admitted to Rasool-e-Akram Hospital, Tehran, Iran (2019 and 2020). The genetic variants were assessed by the Polymerase chain reaction method. The pattern of durability to antibiotics was determined by the disk diffusion system.

Results: Of the 80 isolates that produced ESBL and AmpC beta-lactamase, 75 cases were ESBL producers and 5 cases co-producers of ESBL and AmpC. The most common cultivated strains included Escherichia coli (61.2%) and Klebsiella pneumoniae (31.3%). The highest antibiotic resistance in ESBL producers was related to cefotaxime, trimethoprim-sulfamethoxazole, and cefazolin, and the lowest resistance level was related to colistin, cefotixin, and ceftriaxone. The total frequencies of common genes of ESBL-producing gram-negative bacilli were blaCTX-M (76.0%), blaTEM (46.7%), and blaSHV (26.7%), while multigenic harboring was also observed in 49.3%. The level of drug resistance to Imipenem, Amikacin, and Cefazidime in people who harbored the blaSHV gene was significantly higher than in other cases.

Conclusion: The most common cultivated strains included E. coli and K. pneumoniae. blaCTX-M, blaTEM, blaSHV, and multigenic expression were observed. The detection of the blaSHV gene is associated with increased antibiotic resistance to Imipenem, Amikacin, and Cefazidime.

Keywords: AmpC beta-lactamase, Antibiotic resistance, Extended-spectrum beta-lactamase, Gram-negative bacilli

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1 Introduction

Bacterial durability to antibiotics is an essential scientific issue related to community health as well as hospitals, which has led to the failure of treatments for dangerous infections (1,2). Fast detection of organisms' resistance to antimicrobial is important in clinical laboratories (3). Characterization of the resistance mechanism can facilitate innovation in antimicrobials design. Three general pathways for antibiotic resistance have been identified: A) The generation of beta-lactamases, which break the beta-lactam loop and thus inactivate the beta-lactam antibiotic, B) Lack or decrease in the exposition of outer membrane proteins called OMP in gram-negative pathogens and C) Increased expression of membrane pumps (4-7). ESBL production is an important resistance mechanism that prevents the antimicrobial treatment of gram-negative infections and shows a significant hazard to available antibiotics,
particularly β-lactam antibiotics, including penicillins, cephalosporins, and monobactams (8-10). AmpC β-lactamases preferentially hydrolyze narrow, broad, and expanded-spectrum cephalosporins and cephamycins and abide blockage by clavulanate, sulbactam, and tazobactam. AmpC-producing strains are generally resistant to oxyimino-beta lactams and cephamycins and are sensitive to carbapenems, but decreased porin expression will build such a strain carbapenem-persistent as well (11).

The introduction of carbapenems to the medical world has been the main breakthrough in the remedy of diseases caused by beta-lactam-resistant bacteria. Carbapenems are the Preferred medicine for treating infections caused by penicillin-resistant or cephalosporin-resistant gram-negative bacilli due to their wide range of activity and resistance to hydrolysis by most beta-lactamases (12,13). Resistance to carbapenem has been attributed to various factors such as decreased expression of outer membrane proteins, increased efflux activity, and production of carbapenemase beta-lactamases (14).

According to the Ambler molecular classification, beta-lactamases are divided into four groups: class A, C, and D are serine beta-lactamase, and class B is Metallo-β-lactamase (MBL) (15). Extended-spectrum Beta-lactamases are interdicted by clavulanic acid, sulbactam, and tazobactam and are encoded by different genes (including \(\text{bla}_{\text{SHV}}, \text{bla}_{\text{TEM}}, \text{bla}_{\text{OXA}}, \text{bla}_{\text{PER}}, \text{bla}_{\text{VEB}}, \text{bla}_{\text{GES}}, \text{bla}_{\text{BES}}\)) that can vary between bacteria (16-19). Among the various types of ESBL, the most common subgroups are \(\text{bla}_{\text{CTX}}, \text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV}}\), which are crucial factors in antibiotic resistance in important strains such as Escherichia coli and Klebsiella pneumoniae (20,21).

AmpC Beta-lactamases are encoded by different genes such as \(\text{bla}_{\text{CMY}}, \text{bla}_{\text{FOX}}, \text{bla}_{\text{DHA}}, \text{bla}_{\text{MOX}}, \text{and bla}_{\text{CIT}}\). However, there is little information about the frequency of these plasmid genes and their genetic pattern in Iran.

The outbreak of antibiotic resistance is increasing, and one of the essential resistance mechanisms is the generation of extended-spectrum ESBL and AmpC by gram-negative bacilli. Therefore, determining its prevalence in each region, like hospitals, is crucial. Also, it seems that gene changes of resistant microorganisms can be different in each region, and monitoring the changes can help determine better and more appropriate treatments, especially in more sensitive units such as intensive care units and infectious diseases units. Hence, this research attempted to characterize the outbreak of fast-growing gram-negative bacilli producing ESBL and AmpC and evaluate their common genetic variants.

### 2. Materials and Methods

#### Bacterial Isolates

This cross-sectional research was carried out on 9424 clinical samples (including blood, urine, BAL, trachea, or Shaldon) of patients admitted to different wards of Rasool-e-Akram Hospital (Tehran, Iran) from 2019 to 2020. The samples were sent to the hospital’s laboratory at the request of physicians to detect and isolate various fast-growing gram-negative bacilli. The isolates were recognized using phenotypic techniques.

#### Antibiotic Susceptibility Testing

The pattern of resistance to 8 antibiotics was identified considering Clinical and Laboratory Standards Institute (CLSI) guidelines by disk diffusion method by Rosco Neosensitabs (Rosco Diagnostica, Taastrup, Denmark) (Table 1). Imipenem (10mcg), Meropenem (10mcg), Ampicillin (10mcg), Penicillin (10IU), Aztreonam (30mcg), Cefotaxime (30mcg), Cefoxitin (30mcg), and Ceftazidime (30mcg) were used in this study.

#### Table 1. CLSI guidelines (2020) of current antibiotics against gram-negative bacilli

| Antibiotic   | S(mm or more) | I(mm) | R(mm or more) |
|--------------|---------------|-------|---------------|
| Ceftazidime  | 21            | 18–20 | 17            |
| Cefotaxime   | 26            | 23–25 | 22            |
| Ampicillin   | 17            | 14–16 | 13            |
| Penicillin   | 17            | 14–16 | 13            |
| Imipenem     | 23            | 20–22 | 19            |
| Meropenem    | 23            | 20–22 | 19            |
| Aztreonam    | 21            | 18–20 | 17            |
| Cefoxitin    | 18            | 15–17 | 14            |
If the activity of the above antibiotics, except for Imipenem (meropenem) and Cefoxitin, were inhibited in the culture medium, the presence of ESBL was suspected. In this case, their existence was confirmed by confirmatory tests, which was the use of clavulanic acid (beta-lactamase inhibitor).

**Screening for ESBL-producing Strains**

Suspicious specimens were first cultured on Müller-Hinton agar (Condalab, Spain). Therefore, after suspecting the presence of ESBL, we used a disc containing clavulanic acid and compared the diameter of the inhibitory zone with and without inhibitors so that if the difference was more than 5 mm, ESBL was confirmed (Figure 1).

**Screening for AmpC-producing Strains**

In addition, we were suspected of having AmpC if, in the first stage, all antibiotics, except imipenem (or meropenem), were inhibited in the culture medium, or the second stage, ESBL confirmation tests were negative. Therefore, we used cefoxitin-cloxacillin (CFO-CX) disc. A distinction between the cefoxitin-cloxacillin inhibition zone and the cefoxitin alone zone of ≥4 mm indicated AmpC production (Figure 2).

**Molecular Specification of ESBL and AmpC-beta lactamases Genes**

Finally, special primers were planned for the extracted bacteria to study the genes of bla_TEM, bla_SHV, and blaCTX-M in ESBL, and blaFOX, bla_MOX, bla_CIT, bla_DHA, and bla_EBC for AmpC (Table 2) (22, 23).

### Table 2. Oligonucleotide sequences used in this study

| Target Gene | Primer Sequencing | Annealing Temperature | Product Size(bp) |
|-------------|-------------------|-----------------------|------------------|
| CTX-M       | F:GGT TAA AAA ATC ACT GCG TC  
R:TTG GTG ACG ATT TTA GCC GC | 58 | 863 |
| TEM         | F:CGC CGC ATA CAC TAT TCT CAG AAT GA  
R:ACG CTC ACC GGC TCC AGA TTT AT | 65 | 445 |
| SHV         | F:CTT TAT CGG CCC TCA CTC AA  
R:AGG TGC TCA TCA TGG GAA AG | 58 | 237 |
| CIT         | F:TGG CCA GAA CTG ACA GGC AAA  
R:TTT CTC CTG ACG GTG GCT GGC | 61 | 462 |
| FOX         | F:AAC ATG GGG TAT CAG GGA GAT G  
R:CAA AGC GCG TAA CGG GAT TGG | 63 | 190 |
| MOX         | F:GCT CAA GGA GCA CAG GAT  
R:CAC ATT GAC ATA GGT GTG C | 55 | 517 |
| EBC         | F:TGC GTA AAG CCG ATG TTG CGG  
R:CTT CCA CTG CGG CTG CCA GTT | 63 | 302 |
| DHA         | F:AAC TTT CAC AGG TGT GCT GGG T  
R:CCG TAC GCA TAC TGG CTT TGC | 63 | 405 |
Multiplex PCR (Bio-Rad Thermal Cyclers, Singapore) was utilized to identify the major ESBL and AmpC beta-lactamase genes. Single colonies of each isolate were cultivated, and bacterial cells were bulleted by centrifugation (14000 g for 4 min) after removing supernatants. DNA extraction was carried out by exploiting the microorganism GeneAll KIT (Seoul, Korea). 3 μL of Total DNA (10 ng) was added to every multiplex PCR reaction. The reaction mixture contained 12 μL mastermix (Tempase hot begin, Amplicon, Denmark), 0.7 μL specific primer (10 Pmol), and 5 μL nuclease-free water. In this study, thermal cycling PCR (BIO-RAD) was used according to the following protocol:

The primary denaturation was executed at 95°C for 15 min, followed by 29 cycles of denaturation at 94°C for 30 s, tempering at 54°C for 80 s, extension at 72°C for 1 min and final extension at 72°C for 10 min.

Finally, the Amplified genes were loaded on 2% agarose gel and electrophoresed (Gelldoc, Iran). The PCR product was stained by SYBR stain and visualized by a gel documentation system according to standard procedures.

It is necessary to explain that to carry out this project, no additional sampling was taken from the hospitalized patients. All the steps were taken on the same samples sent to the hospital laboratory at the request of the physicians.

Results of the actuarial assay were reported as mean ± standard deviation (SD) for quantitative variables and were epitomized by frequency (percentage) for classified variables. Contiguous variables were evaluated using t-test or Mann-Whitney test whenever the data did not appear to have normal dispensation or when the presumption of similar variances was breached across the study groups. On the other hand, categorical variables were evaluated using the Chi-square test. A P-value of ≤ 0.05 was considered statistically significant. The statistical software SPSS version 23.0 for windows (IBM, Armonk, New York, USA) was used for the actuarial assay.

3. Results

Bacterial Isolation

In the present research, Out of 9424 samples sent to the laboratory, 288 suspected cases were identified and after confirmatory tests, finally 80 ESBL and AmpC beta-lactamase productive isolates were detected. From these isolates, 75 cases are ESBL producers, and 5 cases are co-producers of ESBL and AmpC.

Because of few cases of co-producers, a total of 75 specimens were extracted and studied. In total, 31 cases (41.3%) were male with mean age 56.8±35.23 years, and 44 cases (58.7%) were female with mean age 55.21±46 years. Specimens were accumulated from various hospital wards (details in Table 3). Also, the extracted samples included urine, BAL, blood, trachea, and Shaldon secretions that the details are described in Table 3.

| Character       | Category                  | Number (percent) |
|-----------------|---------------------------|------------------|
| Gender          | Man                       | 31 (41.3)        |
|                 | Female                    | 44 (58.7)        |
|                 | Emergency                 | 15 (20.0)        |
|                 | Respiratory disease       | 15 (20.0)        |
|                 | Internal medicine         | 9 (12.0)         |
|                 | Infectious disease        | 6 (8.0)          |
|                 | Respiratory ICU           | 7 (9.3)          |
|                 | MICU                       | 5 (6.7)          |
|                 | Gynecology                | 4 (5.3)          |
|                 | Surgery                   | 4 (5.3)          |
|                 | Pediatrics                | 3 (4.0)          |
|                 | post CCU                  | 2 (2.7)          |
|                 | Surgical ICU              | 1 (1.3)          |
|                 | Open heart ICU            | 1 (1.3)          |
|                 | Ophthalmology             | 1 (1.3)          |

Table 3. Characteristics of the studied samples
The types of bacilli identified were *E. coli* in 44 cases (58.7%), *K. pneumoniae* in 25 samples (33.3%), *Pseudomonas aeruginosa* in 3 samples (4.0%), *Enterobacter cloacae* in 2 samples (2.2%) and *Acinetobacter baumannii* in 1 sample (1.3%). The above study and the information obtained were the results of 75 ESBL producers. The frequency of gram-negative bacilli in terms of primary characteristics is listed in detail in Table 4.

### Table 4. Distribution of ESBL gram-negative bacilli according to study characteristics

| Bacillus                  | *E. coli* | Klebsiella | *Pseudomonas aeruginosa* | *Enterobacter cloacae* | *Acinetobacter baumannii* | P-value |
|---------------------------|-----------|------------|-------------------------|------------------------|---------------------------|---------|
| Gender                    |           |            |                         |                        |                           | 0.270   |
| Man                       | 54.8%     | 32.3%      | 9.7%                    | 3.2%                   | 0.0%                      |         |
| Female                    | 61.4%     | 34.1%      | 0.0%                    | 2.3%                   | 2.3%                      |         |
| Mean age, year            | 54±20     | 57±18      | 56±30                   | 72±11                  | 60±10                     | 0.779   |
| Inpatient department      |           |            |                         |                        |                           | 0.001   |
| Emergency                 | 80.0%     | 13.3%      | 6.7%                    | 0.0%                   | 0.0%                      |         |
| Respiratory disease       | 53.3%     | 33.3%      | 6.7%                    | 6.7%                   | 0.0%                      |         |
| Internal medicine         | 55.6%     | 33.3%      | 11.1%                   | 0.0%                   | 0.0%                      |         |
| Infectious disease        | 66.7%     | 33.3%      | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Respiratory ICU           | 14.3%     | 71.4%      | 0.0%                    | 0.0%                   | 14.3%                     |         |
| MICU                      | 20.0%     | 80.0%      | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Gynecology                | 75.0%     | 25.0%      | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Surgery                   | 100%      | 0.0%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Pediatrics                | 100%      | 0.0%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| post CCU                  | 50.0%     | 50.0%      | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Surgical ICU              | 100%      | 0.0%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Open heart ICU            | 100%      | 0.0%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Ophthalmology             | 100%      | 0.0%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Skin disease              | 0.0%      | 100%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Sample type               |           |            |                         |                        |                           | 0.001   |
| Urine                     | 73.7%     | 22.8%      | 1.8%                    | 0.0%                   | 1.8%                      |         |
| Trachea                   | 100%      | 0.0%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
| Blood                     | 0.0%      | 50.0%      | 0.0%                    | 50.0%                  | 0.0%                      |         |
| BAL                       | 8.3%      | 75.0%      | 16.7%                   | 0.0%                   | 0.0%                      |         |
| Shaldon                   | 0.0%      | 100%       | 0.0%                    | 0.0%                   | 0.0%                      |         |
Antibiotic Susceptibility Results

First, there was no difference in the frequency of bacterial strains between men and women based on different age groups. However, isolated Bacillus strains have a great variety in other hospital wards, which are described in detail in Table 4, with the names of each ward. Of course, as detailed in Table 4, the isolated strains also varied according to the type of samples. For example, in urine, the most common isolated strain was E. coli (73.7%), and there were no Enterobacter cloacae, while in blood, E. cloacae (50%) and Klebsiella pneumoniae (50%) were the most common. Regarding antibiotic resistance against ESBL-producing gram-negative bacilli, the frequency of antibiotic resistance in detail is expressed in Table 5. For example, the highest antibiotic resistance against gentamycin is seen in Enterobacter cloacae (100%) and Acinetobacter baumannii (100%), while Pseudomonas aeruginosa had no resistance to gentamycin.

Table 5. Distribution of antibiotic resistance by type of gram-negative bacilli

| Antibiotic         | E. coli | Klebsiella | Pseudomonas aeruginosa | Enterobacter cloacae | Acinetobacter baumannii | P-value |
|--------------------|---------|------------|------------------------|----------------------|-------------------------|---------|
| Gentamycin         | 34.7%   | 76.0%      | 0.0%                   | 100%                 | 100%                    | 0.002   |
| Cefepime           | 77.6%   | 44.0%      | 33.3%                  | 0.0%                 | 100%                    | 0.009   |
| Imipenem           | 8.2%    | 56.0%      | 66.7%                  | 0.0%                 | 100%                    | 0.001   |
| Ampicillin-Sulbactam| 34.7%   | 84.0%      | 66.7%                  | 50.0%                |                         | 0.002   |
| Nitrofurantoin     | 8.2%    | 16.0%      | 0.0%                   | 0.0%                 | 100%                    | 0.049   |
| Amikacin           | 6.1%    | 60.0%      | 66.7%                  | 0.0%                 | 100%                    | 0.001   |
| Cefoxitin          | 0.0%    | 0.0%       | 33.3%                  | 0.0%                 |                         | 0.001   |
| Ciprofloxacin      | 75.0%   | 88.0%      | 66.7%                  | 0.0%                 | 100%                    | 0.050   |
| Cefazolin          | 95.9%   | 52.0%      | 33.3%                  | 0.0%                 | 100%                    | 0.001   |
| Cefotaxime         | 100%    | 100%       | 66.7%                  | 100%                 | 100%                    | 0.001   |
| Trimethoprim-       | 83.7%   | 88.0%      | 100%                   | 100%                 | 100%                    | 0.862   |
| sulfamethoxazole    |         |            |                        |                      |                         |         |
| Piperacillin-       | 4.1%    | 48.0%      | 66.7%                  | 66.7%                | 66.7%                   | 0.001   |
| tazobactam          |         |            |                        |                      |                         |         |
| Ceftiraxone         |         |            |                        |                      |                         |         |
| Ceftazidime         | 4.1%    | 48.0%      | 33.3%                  | 0.0%                 | 0.0%                    | 0.001   |
| Meropenem           | 44.0%   | 66.7%      | 0.0%                   | 0.0%                 | 0.0%                    | 0.001   |
| Cefuroxime          | 2.0%    | 0.0%       | 0.0%                   | 0.0%                 | 0.0%                    | 0.958   |

The total frequencies of common genes of ESBL-producing gram-negative bacilli were blaCTX-M in 76.0%, blaTEM in 46.7%, and blaSHV in 26.7%. While in 14.7%, the type of gene detected was unknown. Also, cases of simultaneous detection of multiple genes were observed in 49.3% of gram-negative ESBL-producing bacilli; of this number, triple gene detection was reviewed in 16.0% (Figures 3, 4, 5, and 6).

In this article, we used positive control to identify blaSHV, blaCTX-M, and blaTEM genes. Still, for blaOXA, blaMOX, blaGTT, blaEBC, and blaFOX genes, positive control species could not be found because no study was done previously. The frequency of genes by sex, age, type of bacilli, and sample evaluated are described in Table 6.
Table 6. Frequency of common genes of ESBL gram-negative bacilli according to study characteristics

| GENES     | CTX-M | TEM | SHV | unknown | Multi |
|-----------|-------|-----|-----|---------|-------|
| Total     | 76.0% | 46.7% | 26.7% | 14.7%   | 49.3% |
| Gender    |       |     |     |         |       |
| Man       | 64.5% | 48.4% | 22.6% | 25.8%   | 47.4% |
| Female    | 84.1% | 45.5% | 29.5% | 6.8%    | 50.0% |
| Mean age, year | 56.56 | 54.97 | 58.86 | 55.40   | 55.39 |
| Sample type |       |     |     |         |       |
| Urine     | 77.2% | 50.9% | 17.5% | 14.0%   | 47.4% |
| Trachea   | 0.0%  | 100% | 0.0% | 0.0%    | 0.0%  |
| Blood     | 100%  | 0.0% | 0.0% | 0.0%    | 0.0%  |
| BAL       | 66.7% | 33.3% | 58.3% | 25.0%   | 58.3% |
| Shaldon   | 100%  | 100% | 100% | 0.0%    | 100%  |
| Type of bacilli |       |     |     |         |       |
| E. coli   | 77.3% | 45.5% | 9.1% | 13.6%   | 43.2% |
| Klebsiella| 72.0% | 52.0% | 60.0% | 20.0%   | 64.0% |
| Pseudomonas aeruginosa | 66.7% | 33.3% | 33.3% | 0.0%   | 33.3% |
| Enterobacter cloacae | 100% | 0.0% | 0.0% | 0.0%    | 0.0%  |
| Acinetobacter baumannii | 100% | 100% | 0.0% | 0.0%    | 100%  |

The relationship between gram-negative bacilli’s common genes and antibiotic resistance is illustrated in Table 7.
Table 7. Frequency of antibiotic resistance according to common genes of gram-negative bacilli

| Antibiotic                      | CTX-M | TEM  | SHV  | Unknown | Multi |
|---------------------------------|-------|------|------|---------|-------|
| Gentamycin                      | 45.9% | 45.0%| 61.9%| 20.0%   | 45.2% |
| Cefepime                        | 68.9% | 60.0%| 61.9%| 40.0%   | 66.7% |
| Imipenem                        | 23.0% | 27.5%| 42.9%| 0.0%    | 23.8% |
| Ampicillin-Sulbactam            | 47.5% | 42.5%| 61.9%| 0.0%    | 42.9% |
| Nitrofurantoin                  | 9.8%  | 12.5%| 9.5% | 0.0%    | 9.5%  |
| Amikacin                        | 23.0% | 35.0%| 42.9%| 20.0%   | 28.6% |
| Cefoxitin                       | 1.6%  | 2.5% | 0.0% | 0.0%    | 2.4%  |
| Ciprofloxacin                   | 75.4% | 71.8%| 81.0%| 100%    | 75.6% |
| Cefazolin                       | 82.0% | 80.0%| 66.7%| 100%    | 83.3% |
| Cefotaxime                      | 98.4% | 67.5%| 100% | 100%    | 97.6% |
| Trimethoprim-sulfamethoxazole   | 88.5% | 92.5%| 85.7%| 100%    | 92.9% |
| Piperacillin-tazobactam         | 16.4% | 20.0%| 33.3%| 0.0%    | 16.7% |
| Ceftiraxone                     | 0.0%  | 0.0% | 0.0% | 0.0%    | 0.0%  |
| Ceftazidime                     | 16.4% | 17.5%| 33.3%| 0.0%    | 0.0%  |
| Meropenem                       | 13.1% | 15.0%| 28.6%| 0.0%    | 14.3% |
| Cefuroxime                      | 1.6%  | 0.0% | 0.0% | 0.0%    | 0.0%  |

First, there was no observed association between the detection of any of the blaCTX-M, blaTEM, or blaSHV genes, as well as the detection of a multigenic pattern with resistance to gentamicin, nitrofurantoin, ciprofloxacin, cefazolin, cefotaxime, trimethoprim-sulfamethoxazole, piperacillin-tazobactam, or cefuroxime. Regarding imipenem, the drug resistance was remarkably greater in those who harbored the blaSHV gene. Drug persistence was also remarkably greater in those who harbored the blaSHV gene regarding amikacin. Also, the drug resistance was significantly greater for ceftazidime in those who detected the blaSHV gene. It is necessary to explain that in co-producers of ESBL and AmpC, blaCTX-M gene was the main gene, in which in 3 cases is accompanied by CTX-M and TEM, in 1 case with blaCTX-M and another case is associated with blaTEM. In these cases, resistance to cefotaxime, trimethoprim-sulfamethoxazole, ciprofloxacin, and ceftazidime was common, but these relations were not statistically significant.

4. Discussion

A large group of genes associated with ESBL and AmpC beta-lactamases expression is involved in inducing antibiotic resistance in various bacteria. Identifying these genes and assessing their detection will be crucial in determining the pattern of antibiotic resistance. In other words, a significant proof for the change in the drug resistance of bacteria (intensification or reduction of resistance) is the change and occurrence of mutations in these genes and the change in their expression. Therefore, detection of these genes can be important at the clinical level and facilitate the traceability of changes in antibiotic resistance in different parts of the hospital. The current research identified the frequency of fast-growing gram-negative bacilli producing ESBL and studied their common genetic changes in Rasool-e-Akram Hospital. In this evaluation, ESBL-producing gram-negative Bacilli strains were first identified in various blood, urine, trachea, Shaldon, and BAL samples by cultivating in the mentioned methods.

Remarkable results in the present study were:

A) The most common cultivated strains were E. coli (58.7%), K. pneumoniae (33.3%), P. aeruginosa (4.0%), Enterobacter cloacae (2.7%), and Acinetobacter baumannii (1.3%);

B) Regarding antibiotic resistance evaluated in different sections, the highest antibiotic resistance was related to cefotaxime with a resistance of 98.8%, trimethoprim-sulfamethoxazole with 86.2%, cefazolin with 77.5%, ciprofloxacin with 76.0%, and cefepime with the resistance of 63.8%. In this regard, the lowest level of resistance was related to colistin with no
identified resistance, cefoxitin and ceftriaxone with 1.2% resistance;

C) The communication between detecting some bacterial genes and antibiotic resistance was notable. First, the level of drug resistance to imipenem in people who harbored the \textit{bla}\textsubscript{SHV} gene was significantly higher than in the cases that did not harbor it. Also, similarly, drug resistance to amikacin and ceftazidime was significantly higher in those who detected the \textit{bla}\textsubscript{SHV} gene. Therefore, studying the role of the \textit{bla}\textsubscript{SHV} bacterial gene in increasing antibiotic resistance to drugs such as imipenem, amikacin, and ceftazidime, which are widely used drugs in various sectors, suggests that the \textit{bla}\textsubscript{SHV} bacterial gene evaluation can be routinely used to evaluate the antibiotic resistance pattern of ESBL-producing gram-negative bacilli.

Based on the different characteristics of different communities and the genomic pattern of bacteria in other communities, a different expression of bacterial genes was obtained simultaneously with antibiotic resistance, which could be specific to that community. In the study by Gundran \textit{et al.} (24), the highest prevalence was related to the \textit{bla}\textsubscript{CTX-M-1} gene, with a prevalence of 72.4%, followed by the \textit{bla}\textsubscript{CTX-M-2} gene. The \textit{bla}\textsubscript{TEM} and \textit{bla}\textsubscript{SHV} genes frequency were 57.9% and 27.5%, respectively. Simultaneous expression of \textit{bla}\textsubscript{CTX-M} was observed in 73.5% of patients, utterly different from the values obtained in the existing research. In the study by Pishiwan \textit{et al.} (25), of the ESBL-producing \textit{E. coli} cases, 81% displayed the \textit{bla}\textsubscript{TEM} gene, 16.2% the \textit{bla}\textsubscript{SHV} gene, and 32.4% the \textit{bla}\textsubscript{CTX-M} gene. Similarly, 64.7% showed \textit{bla}\textsubscript{TEM} gene, 35.2% \textit{bla}\textsubscript{SHV} gene, and 41.1% \textit{bla}\textsubscript{CTX-M} gene in \textit{K. pneumoniae} samples. In the study of Farzi \textit{et al.} (26), from 100 urine samples, 21 ESBL producer cases (21%) were detected, and the presence of \textit{bla}\textsubscript{CTX-M} and \textit{bla}\textsubscript{TEM} genes in the isolates were 21% and 20%, respectively.

In the study of Roshdi Maleki \textit{et al.} (27), of 120 strains of \textit{K. pneumoniae}, 71 (59.2%) were established for ESBL. The \textit{bla}\textsubscript{TEM} and \textit{bla}\textsubscript{SHV} ESBLs were identified in 35 (49.3%) and 31 (43.7%) strains respectively. Finally, the co-existence of \textit{bla}\textsubscript{TEM} and \textit{bla}\textsubscript{SHV} was detected in 5 (7%) isolates. In a study by Bajpai \textit{et al.} (28) in India, genes related to beta-lactamase production were examined, including \textit{bla}\textsubscript{CTX-M-M} (48.7%), \textit{bla}\textsubscript{TEM} (7.6%), and \textit{bla}\textsubscript{SHV} (1.5%). In a study by Ghaima \textit{et al.} (29) that investigated ESBL genes in \textit{Acinetobacter baumannii} isolates, \textit{bla}\textsubscript{CTX-M-M}, \textit{bla}\textsubscript{TEM}, and \textit{bla}\textsubscript{SHV} genes were discovered in 45%, 75%, and 27.5%, respectively. In a study by Kumars Amini \textit{et al.} (30) in Iran, from 200 diarrhea specimens, 60 (30%) \textit{Shigella sonnei} strains were found. The molecular test results showed that 40 (66.6%) and 33 (55%) of the strains harbored the \textit{bla}\textsubscript{CTX-M-15} and \textit{CMY} genes, respectively.

The \textit{bla}\textsubscript{CTX-M-2} gene was not identified in any of the specimens. Also, in the study of Mubarak Saif Alfaresi et al. (31) in the UAE, out of 240 cases of ESBL, 228 cases carried the ESBL \textit{bla} gene. Also, 87% of the strains carried the \textit{bla}\textsubscript{CTX-M-15} gene, and the \textit{bla}\textsubscript{SHV-28} gene was identified in 13% of these strains. Kooshesh \textit{et al.} (32) found that in ESBL and AmpC positive isolates in Kerman in 2015, 76.1% were positive for \textit{bla}\textsubscript{TEM}, 14.2% positive for \textit{bla}\textsubscript{OXA}, and 2.3% positive for \textit{bla}\textsubscript{SHV}, and none of the isolates were found positive for \textit{bla}\textsubscript{PER}. The outbreak of ESBL, AmpC, \textit{bla}\textsubscript{TEM}, and \textit{bla}\textsubscript{OXA} in inpatients isolates was 7.2%, 0.2%, 37.2%, and 8.5%, respectively, which differed from our study terms of genotype and destination.

As it is evident, in different societies and even in a society with different geographical conditions, both the rate of antibiotic resistance and the expression of bacterial genes associated with these resistances have been reported quite differently, which can be related to both the genomic conditions associated with that community and differences in the techniques used to assess genomic expression.

5. Conclusion

In conclusion, in our study population, first of all, we faced high antibiotic resistance to cefotaxime, trimethoprim-sulfamethoxazole, cefazolin, ciprofloxacin, and cefepime. Also, the prevalence of multi-antibiotic resistance in our society is high. In terms of identifying bacterial genes associated with ESBL-producing gram-negative bacilli, the outbreak of common ESBL-producing gram-negative bacilli genes was \textit{bla}\textsubscript{CTX-M} in 76.0%, \textit{bla}\textsubscript{TEM} in 46.7%, and \textit{bla}\textsubscript{SHV} in 26.7% of bacilli. Also, cases of simultaneous harboring of multiple genes were observed in 49.3% of gram-negative ESBL-producing bacilli. Finally, \textit{bla}\textsubscript{SHV} gene detection will be associated with increased antibiotic resistance to imipenem, amikacin, and ceftazidime.

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Conflict of Interest

The authors declared no conflict of interest.

Authors Contribution

Study Conception, Design, Data Analysis and Interpretation of Results: Dr. Pegahas Babaeiheidarian, Majid Mehdinejad. Design and Interpretation of Results: Dr. Ali Zare-Mirzaei. Data Collection: Roya Mokarimejad, Ensieh Jafari.

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