Detection of ESBL and MBL Antibiotic Resistance Genes in 100 Clinical Isolates of Escherichia Coli by Multiplex PCR

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Abstract

Bckground: Increasing use of beta-lactam antimicrobials in the treatment of bacterial infections has increased resistance against them.

Objectives: This study aimed to investigate the patterns of antibiotic susceptibility to beta-lactam antibiotics and to investigate the presence of beta-lactamase and Metallo-beta-lactamase genes blaKPC, blaTEM, blaAmpc, blaIND, blaSIM, and blaGIM in clinical specimens of *Escherichia coli*.

Methods: In this study, 100 urine samples were collected from different wards of hospitals and treatment centers in the west of Tehran province, and 100 strains of *Escherichia coli* were confirmed by biochemical tests. In the next step, a susceptibility test was performed on 3 selected antibiotics. Then, using the Combine Disk Test method, ESBL and MBL strains were identified. Finally, using the multiplex PCR method, the strains producing KPC, TEM, Ampc, IND, GIM, and SIM enzymes were identified.

Results: In this study, the highest resistance of strains to cefotaxime was observed. n = 52 (52%) and their highest sensitivity to imipenem was seen n = 95 (95%). Also, n = 53 (53%) of the samples had ESBL genes. Also, 41 isolates (77%) of the studied strains contained the blaTEM gene, 12 isolates (23%) of the strains contained the blaAmpc gene and 20 isolates (38%) of the strains contained blakpc gene. Also, n= 19 (19%) of the samples had MBL genes. Also, 4 isolates (21%) of the strains contained the IND gene, 4 isolates (21%) of the strains contained the GIM gene, 7 isolates (37%) contained the SIM gene.

Conclusion: Due to the high percentage of resistance to third-generation cephalosporins, careful antibiogram testing before antibiotic administration in infections caused by ESBL and MBL-producing organisms is an unavoidable necessity. Therefore, by quickly and correctly identifying the pattern of antibiotic resistance, the physician will be able to select the appropriate antibiotic therapy and prevent the spread of antibiotic resistance.

Introduction

One of the most important health problems in many parts of the world is antibiotic-resistant nosocomial infections caused by various bacteria, including *Pseudomonas aeruginosa*, *E. coli*, and *Acinetobacter baumannii*, which produce broad-spectrum beta-lactamases and Metallo-β-lactamases, the prevalence of which is threatening serious for human health. So that even choosing the right option for the treatment of severe infections has become difficult (1). The World Health Organization recently officially published a list of antibiotic-resistant bacteria that threaten human life, consisting of 12 families:

The first, critical priority includes: 1- *Acinetobacter baumannii*, resistant to carbapenem antibiotic 2- *Pseudomonas aeruginosa*, resistant to carbapenem antibiotic 3- Enterobacteriaceae, resistant to carbapenem antibiotic

Second, severe priority includes 1. *Enterococcus faecium*, resistant to vancomycin 2. *Staphylococcus aureus*, resistant to methicillin and vancomycin 3. *Heliobacter pylori*, resistant to clarithromycin 4. Fluoroquinolone resistant *Campylobacter* 5. Fluoroquinolone resistant *Salmonella*, 6. *Neisseria gonorrhoeae* resistant to fluoroquinolones and cephalosporins

Third, moderate priorities include 1. *Streptococcus pneumoniae*, resistant to penicillin 2. *Haemophilus influenza*, resistant to ampicillin 3. Shigella, resistant to fluoroquinolone (2).

One of the worrying resistances of the strains containing Metallo beta-lactamase (MBL) and broad-spectrum beta-lactamase (ESBL) (3).

Studies in Iran have shown that the multiple resistance of strains isolated from nosocomial infections has reached a worrying level and their frequency is increasing and even witnesses of extensively broad-spectrum resistance (XDR) and pan drug-resistant (PDR) with global resistance (4).

For the treatment of infections caused by beta-lactam-resistant strains, carbapenems (imipenem, meropenem) are one of the alternatives, but in recent years, carbapenem resistance has been abundantly reported in strains isolated from clinical specimens. One of the important reasons for this resistance is the production of carbapenem hydrolyzing enzymes such as MBL and ESBL (5).

Increasing use of beta-lactam antibiotics in the treatment of bacterial infections has increased resistance against them (6).

At present, one of the problems in the treatment of nosocomial infections is enzymatic resistance to broad-spectrum beta-lactamases (ESBL) and Metallo-beta-lactamases (MBL) among clinical isolates, especially *Escherichia coli*. The choice of effective treatment is difficult (7).

Due to the lack of familiarity of physicians with ESBL and MBL types and their interpretation, the identification of these genes can provide a suitable treatment model (8). Recognition of each type of ESBL and MBL is usually associated with its treatment protocol. In many cases, delay in treatment will lead to irreparable damage to the patient (9). In this study, due to the high prevalence of ESBL and MBL drug resistance, especially therapeutic failures in the treatment of UTI infections, it seems necessary to identify common ESBL and MBL strains.

E. coli can cause disease when it is colonized in tissues that do not have this bacterium as their natural flora. *E. coli* enters the bloodstream, and *E. coli* is the most common gram-negative bacterium that causes sepsis.

This study aimed to identify ESBL and MBL antibiotic resistance genes in 100 clinical isolates of Escherichia coli by Multiplex PCR.

Methods

Sample collection

To conduct this study, 100 clinical samples isolated from patients with urinary tract infections were collected during 1 month by referring to hospitals and medical centers in the west of Tehran province and were transferred to the university's microbiology laboratory.

Morphology, Staining, and Biochemical tests

Differential culture media were used to identify samples by genus. For this purpose, after culture on EMB medium and observation of green polished colonies on this medium, gram staining was performed and after confirmation of gram-negative bacilli, diagnostic tests Simmon Citrate, MR-VP, TSI, SIM, and IMVIC were continued.

Determination of Antibiotic Resistance

For antibiogram, the antibiotic discs of ceftazidime (30 µg), cefotaxime (30 µg), and imipenem (10 µg) (discs of Padten Teb Tehran-Iran) were used, plates incubated for 18-24 hours at room 37 ° C and then the diameter of the non-growth zone around the discs was measured with a standard ruler. Antibiogram results are evaluated according to table 1 (CLSI 2020).

Table 1: Bacterial susceptibility to antibiotic discs

The diameter of the growth inhibition zone			Abbreviation	Antibiotic
Sensitive(mm)	Intermediate(mm)	Resistant(mm)		
21≥	18-20	≤17	CAZ	Ceftazidime
26≥	23-25	≤22	CTX	Cefotaxime
23≥	20-22	≤19	IPM	Imipenem

Detection of broad-spectrum beta-lactamases (ESBL)

The main strategy for detecting ESBL-producing bacteria is to use a cephalosporin as an identifier for the possible screening of ESBL-producing bacteria and then to find a synergism between cephalosporin/clavulanate that lacks the distinction between ESBL-producing and sample-free.

Combine Disk test for ESBL phenotype

For the diagnosis of ESBL, use the antibiotic discs of ceftazidime (30 µg), ceftazidime/clavulanate (beta-lactamase inhibitor) (30.10 µg) and cefotaxime (30 µg), cefotaxime/clavulanate (beta-lactamase inhibitor). A single colony of fresh bacterial culture was added to 5 ml of sterile distilled water on LB agar medium, and after its turbidity reached 0.5 McFarland standard, some sterile bacterial suspension was applied three times at a 60^{oc} to each other. Cultured on MHA medium, then the antibiotic disks of ceftazidime (30 µg), ceftazidime/clavulanate (30.10 µg) and cefotaxime (30 µg), cefotaxime/clavulanate

(30.10 µg) were placed on the culture plate at appropriate intervals. After 24 hours of incubation at 37 ° C, the diameter of the growth inhibition zone around the disks is measured with a ruler. At this stage, each plate that shows the cephalosporin-related growth inhibition zone in the presence of clavulanate is synergistic and is reported as producing the ESBL phenotype. Then, to confirm the accuracy of the Combine Disk method, the zone of detecting antibiotics in the absence of clavulanate and its presence was measured and compared. Samples that showed an increase of 5 ≤ mm in the absence of growth in the presence of clavulanate compared to the absence of clavulanate as producers of ESBL phenotype were recorded (Table 2).

Table 2: Specifications of ESBL antibiotic test disc

µg/disk	Abbreviation	Antibiotic
30	CAZ	Ceftazidime
30	CTX	Cefotaxime
30/10	CZA	Ceftazidime/clavulanate
30/10	CTC	Cefotaxime / clavulanate

Detection of metallo-beta-lactamase (MBL)

For the detection of MBL, a combined disc is used, the EDTA compound is used inside the disc. First, a single colony of fresh bacterial culture was added to 5 ml of sterile distilled water on LB agar medium, and after its turbidity reached 0.5 McFarland standard, some sterile bacterial suspension was applied three times at a 60-degree angle by the sterile swab. Imipenem and Imipenem / EDTA antibiotic disks were placed at a suitable distance on the bacterial culture plate. After 24 hours of incubation at 37 ° C, the diameter of the growth inhibition zone around the disks with the ruler was measured. If the diameter around the imipenem / EDTA disc increased by 7 mm compared to the imipenem disc alone, it indicates the presence of the enzyme metallo-beta-lactamase in the test isolate (Table 3).

Table 3: Specifications of MBL antibiotic test discs

µg/disk	Abbreviation	Antibiotic
10	IMP	imipenem
750/10	IEH	EDTA/ imipenem

Molecular Detection

Bioinformatics Studies

The target gene and the appropriate primers were used to detect resistance genes according to scientific studies and bioinformatics software. Forward and Reverse primers were blasted on the NCBI website, which showed the suitability of the selected primer pair. In the next step, the primer pair was examined in Oligo Analyzer software and their initial approval of the primer pair in the online software In silico PCR amplification was evaluated.

Primer sequences for ESBLand MBL were indicated in Table 4,5.

Table 4: ESBL dtection primers

gene	F	R	Tm	Amplicon size
TEM	GAGGACCGAAGGAGCTAACC	TTGCCGGGAAGCTAGAGTAA	60	188
AmpC	CTCGACCTCGCGACCTATAC	CTGCCACTGGCGGTAGTAGT	60	102
KPC	CAGCTCATTCAAGGGCTTTC	GTCCAGACGGAACGTGGTAT	60	283

Table 5: MBL detection primers

gene	F	R	Tm	Amplicon size
IND-6	AGGGCATACTGCCGATAATG	CATGGTTTTAGGCCATTGCT	60	139
GIM	GTGCTGGGCATACAGAGGAT	AACTTCCAACTTTGCCATGC	60	210
SIM	GCCCAGGACACACTCAAGAT	CGCATTGTTAGAGGCACTCA	60	327

DNA extraction

DNA extraction was performed according to the Promega kit.

Multiplex PCR

The PCR multiplex method is a type of PCR in which two or more target sequences can be amplified using two or more pairs of primers in a single reaction. This study aimed to identify three ESBL (AmpC, KPC, TEM) and MBL (GIM, SIM, IND) genes simultaneously, which are identified in two separate groups by the Multiplex PCR method.PCR reaction was performed to evaluate the target genes with the standard reaction conditions in a volume of 20 µl. The final concentration and volume of PCR material are given in table 6.

Table6: PCR reactions used in the PCR process

Compound	Concentration
Master Mix (1x)	12.5 µl (1x)
F Primer Gene1(0.1-1 µm)	1 µl (10 µm)
R Primer Gene1 (0.1-1 µm)	1 µl (10 µm)
F Primer Gene2 (0.1-1 µm)	1 µl (10 µm)
R Primer Gene2 (0.1-1 µm)	1 µl (10 µm)
F Primer Gene3 (0.1-1 µm)	1 µl (10 µm)
R Primer Gene3(0.1-1 µm)	1 µl (10 µm)
Template DNA	1 µl (20pg)
Sterile Deionized Water	5.5 µl
Total Volume	25 µl

The PCR reaction in the Corbett device was performed in 35 cycles according to the conditions in table 7.

Table7: PCR cycles

PCR cycles	Temperature	Time (second)	Cycles
Initial Denaturation	95°C	300	1
Denaturation	95°C	30	35
Annealing	60°C	45	35
Extention	72°C	40	35
Final Extention	72°C	300	1

Gel Electrophoresis

Electrophoresis gel was used to observe and confirm PCR products.

Results

Antibiogram results

According to the diameter standards of the growth inhibition zone for the antibiotics used, the percentage of sensitive, resistant, and intermediate strains was calculated. The results of antibiogram testing of isolated *Escherichia coli* clinical strains are shown in Figure 1.

Evaluation of the results of broad-spectrum beta-lactamase detection

The results obtained by the Combine Disk diffusion method showed that among the 100 isolates studied, %53 isolates produced broad-spectrum beta-lactamase. (Figure 2)

Evaluation of metallo-beta-lactamase detection results

The results obtained by the Combine Disk diffusion method showed that among the 100 isolates studied, %19 isolates produced metallo-beta-lactamase. (Figure 3)

Results of Multiplex PCR

Multiplex PCR experiments on all samples that were confirmed by Combine Disk method of ESBL and MBL production using 6 pairs of specific primers for ESBL (TEM, Ampc, KPC) genes and (IMP, VIM, IND-6) MBL were separately done.

Evaluation of TEM, Ampc, KPC (ESBL) genes in *Escherichia coli* clinical isolates

Following amplification of TEM, Ampc, and KPC genes by Multiplex PCR, the product of 283, 102, 188 bp was identified in only 53 *Escherichia coli* isolates confirmed by the Combine Disk method. (Figure 4-10). In this study, in 53 isolates, 3 isolates 27, 28, 31 did not match with the Combine Disk results. Clinical isolates carrying TEM, Ampc, and KPC genes in this study are listed in Table (4-6).

Evaluation of MBL (IND, GIM, SIM) genes in *Escherichia coli* clinical isolates

Following amplification of IND, GIM, and SIM genes by Multiplex PCR method, the product of 137,210,327 bp, respectively, was identified in only 19 *Escherichia coli* isolates confirmed by the Combine Disk method.

As shown in the diagram, 2 of the *19 Escherichia coli* isolates have two GIM / SIM genes and 2 of the 19 isolates have three IND / GIM / SIM genes simultaneously.

Discussion

Escherichia coli is a common opportunistic pathogen in nosocomial infections. Increasing use of beta-lactam antibiotics has led to antibiotic resistance in this bacterium, which is usually due to the production of beta-lactamase enzymes. The production of beta-lactamase enzyme in bacteria, especially in *Escherichia coli*, has caused many problems for the treatment of patients.

Organisms that carry these genes increase morbidity and mortality among individuals, which will pose a serious threat to society as the growing trend of resistance develops. (10).

In all efforts to reduce antibiotic resistance, three main factors should be considered, namely the timely identification of isolates producing beta-lactamase and broad-spectrum enzymes, appropriate treatment measures.

Arbitrary, improper, and excessive use of antibiotics in medicine has caused resistant strains. In the present study, *Escherichia coli* 100 isolates collected from patients with urinary tract infections. Out of 100 isolates, 53% had ESBLs and 19% had MBL.

77% of isolates contained TEM gene and 23% contained Ampc gene and 38% contained KPC gene. 15% of isolates contained both TEM and Ampc genes, 15% of isolates contained both TEM and KPC genes, and 15% of isolates contained all three TEM, Amp, and KPC genes.

21% of isolates contained IND gene, 21%of isolates contained GIM gene and 37% of isolates contained SIM gene. 11% of isolates contained both GIM and SIM genes and 11%of isolates contained all three genes IND, GIM, and SIM.

According to the results of the present study, the highest and lowest antibiotic resistance was observed against cefotaxime (52%), ceftazidime (27%), and imipenem (2%), respectively.

In a study conducted by Mobasher and colleagues in Tabriz, it was found that out of 41 isolates of *Escherichia coli*, 98% reported the isolates as ESBL (11).

In the study of Manouchehri et al. In 1994, 200 urine samples were collected from the infectious and nephrology wards of Razi hospitals in Ghaemshahr and Imam Khomeini hospitals in Sari, and only 120 *Escherichia coli* isolates were isolated. Of these, 55% were broad-spectrum beta-lactamase-producing strains. The prevalence of antibiotic resistance is very high compared to the present study.

In a 2013 study by Mahmoud Asaf Habib in Pakistan, he noted an increase in ESBL in *Escherichia coli* from 34% in 2005 to 60% in 2009, with resistance to cefotaxime and ceftazidime above 85%, especially in urine samples. The increase is significant and has increased from 10–65%. (12) The study of Manouchehri and Asf Habib is in line with our study in terms of the frequency of isolated ESBLs. But in terms of resistance to cefotaxime and ceftazidime antibiotics, they show high resistance compared to the present study.

In a study conducted by Shahcheraghi in 2007, 45% of ESBL positive samples were resistant to cefotaxime and 48% to ceftazidime (13). In the study of Mashouf et al. In 2013, resistance to ciprofloxacin 17% and imipenem 0%: was reported (14).

In a 2005 study by Kadder et al. sensitivity to imipenem antibiotics was 92% and ciprofloxacin was 59% (15).

In a study conducted between March 2012 and December 2012 at Al-Zahra Hospital in Isfahan by Reza Moayedtia et al., *Escherichia coli* isolates 0.3% contained MBL (16).

The frequency of MBL genes in the study of Lozaro et al. was reported to be 0.7%. (17). In a study conducted in 2011 by Sara Abdollahi Khairabadi, et al. 87% were identified as sensitive to imipenem (18).

In a study conducted between 2009 and 2011 by Fereshteh Shahcheraghi et al. In Tehran, out of 244 isolates of *Escherichia* coli, 9 isolates (4%) to meropenem, 1 isolate (0.4%) to imipenem, two isolates (0.8%) were reported to be resistant ertapenem (19). In a study conducted between March 2012 and December 2012 in Al-Zahra Hospital in Isfahan by Moayednia et al., 1% were reported to be resistant to imipenem (20).

The prevalence of antibiotic resistance of imipenem in our studies and others is almost equal. According to similar studies with the present studies, the prevalence of isolated ESBLs and antibiotic resistance of cefotaxime and ceftazidime in *Escherichia coli* is increasing. They are important in infections. They require strict therapeutic supervision in the use of antibiotics. The frequency of MBL isolates in our study compared to other studies indicates an increase in the prevalence, which is very worrying in the antibiotic treatment of *Escherichia coli* infection.

In the study of Yazdi et al. In 1989, out of 246 isolates studied, 47% were ceftazidime resistant isolates and 39% were cefotaxime resistant isolates. Also, 44% of isolates were ESBL. The bla^{TEM} gene was found in 87% of ESBL isolates (21). Mohammad Mehdi Sultan Dalal in 2010 showed that out of 200 *Escherichia coli* isolates, 64% were ESBL, of which 58% contained TEM gene (22). In 2007 in Valiasr Hospital of Tehran, among 76 clinical samples of *E. coli*, 60% of isolates contained TEM gene (23). Masjedian showed that out of 148 strains *E.coli* 85% of the isolates contained the TEM gene (24). In a study by Mir Salehian, it was found that out of 33 isolates of *Escherichia coli*, 39% of the isolates contained beta-lactamase TEM gene (25).

Similar studies in Turkey showed that 53% of *Escherichia coli* isolates had the TEM plasmid (26).

Comparison of the results of the present study with the other studies indicates the high prevalence of beta-lactamase genes, especially TEM type in *Escherichia coli* isolates, so it is necessary to identify this type of resistance using molecular methods along with phenotypic methods. In the study of Dokht Shamstalab et al. in 1995, 100 clinical isolates of *Escherichia coli* were collected from hospitals in Kermanshah. After further examination of these 44 isolates by multiplex PCR, it was determined that 44% of them had the AmpC beta-lactamase gene.

In a 2010 study of 909 gram-negative bacteria by Manoharan et al. in India, 312 isolates were resistant to cefoxitin and used the combined disk method for phenotypic confirmation, which considered 36% AmpC producer. After PCR, 42% had the AmpC gene (27). In 2012, Tanja et al. conducted a study on 100 *Escherichia coli* isolates in India, which showed that 59 AmpC-producing isolates were considered to have 57% AmpC gene after PCR (28).

In 2013, Hackman and colleagues tested 400 isolates of the bacterium in the country, 50 of which were resistant to cefoxitin. Of the 50 cefoxitin-resistant isolates, 10% produced AmpC beta-lactamase, meaning that only 1% of the 400 isolates produced AmpC beta-lactamase (29).

The differences between the results of this study and other studies may be due to differences in the geographical area and genetics of individuals, differences in bacterial strains, as well as differences in the type of antibiotics prescribed or how they are used.

In the researches of Soltan Dallal et al and Mansouri (30) which were performed on *Escherichia coli* isolates in Tehran in 2010 and 2007, the prevalence of AmpC genes was 3% and 6%, respectively. It was reported that their results are significantly different from the present study and it can be concluded that the prevalence of resistance genes in these bacteria is increasing over the years and this issue should be further investigated. In addition to the difference in the geographical area, the reason is that doctors mistakenly prescribe patients with arbitrary and unprincipled consumption or other reasons.

ESBLs genes are associated with the development of multiple resistances to other antibiotics, so that the occurrence and spread of various antibiotic genes, especially as multidrug, due to the increased resistance of ESBLs, has created many problems for the treatment of infections caused by them. According to our research and others, this increase in ESBL-producing strains is worrying and the need for the annual review of such research will change the treatment aid of patients and the treatment strategy in hospitals.

Based on the results of this study and other studies, it seems that the widespread and uncontrolled use of broad-spectrum beta-lactam antibiotics and transmission by hospital staff and patient-to-patient transmission has led to an increasing number of ESBL-producing strains in the community. Extensive use of beta-lactam antibiotics, especially cephalosporins, has increased the prevalence of broad-spectrum beta-lactamase (ESBL) enzymes in *Escherichia coli*. It is effective in treating resistant infections and preventing the increase of antibiotic resistance, as well as performing antibiogram tests before prescribing antibiotics to prevent the indiscriminate use of beta-lactam antibiotics.

In a study conducted by Tawfik Abd Motaleb from 2011 to 2012 on 105 isolates containing metallo-beta-lactamase isolated from the ICU of Shams Teaching Hospital. Eleven isolates belonged to *Escherichia coli*: 1 isolate (9%) contained blaGIM and 2 isolates (18%) contained bla SIM (31).

In a 2013 study of 230 clinical isolates from 2007 to 2011, 50 clinical isolates including *Pseudomonas spp.*, Enterobacteriaceae (*Enterobacter cloacae*), *Klebsiella oxytoca*, *Serratia marcescens*, *Escherichia coli* were positive for the blaGIM-1 gene. Of these, only one *Escherichia coli* isolate isolated from blood culture had the blaGIM-1 gene (32).

A 2019 study was performed on 88 patients with sepsis. *E. coli* isolates were detected by gram staining and biochemically. The polymerase chain reaction was performed for *E. coli* genes producing ESBL and carbapenemase. Of 88 patients with sepsis, 49 and 30 strains had ESBL and carbapenemase. Of 30 carbapenemase-producing genes in E. coli had 2 isolates (7%) bla SIM and 1 isolate (3%) blaGIM and no isolates were bla KPC and 2 isolates (7%) had both bla SIM and blaGIM genes (33).

There are few studies about the prevalence of IND GIM, SIM, the gene in *Escherichia coli*. However, these studies show that these genes are more prevalent in *Escherichia coli*. Infection control measures are needed, including management of antibiotic use and rapid identification of resistant isolates. The genes encoding MBLs are located on class I integrons and transmissible plasmids; therefore, the study of these enzymes is essential to rapidly identify resistant strains to prevent their spread.

The frequency of different prevalence is probably due to differences in the type of clinical isolates, geographical location according to the results of the present study, and comparison with other similar studies. The frequency and prevalence of antibiotic resistance are different according to geographical areas, so maybe this is the reason for the differences between the results of this study and the other studies. The differences between the results of this study and other studies are evidence of differences in the frequency and prevalence of antibiotic resistance between different countries, different hospitals and hospital wards, and even between individuals. It can be related to the rate of antibiotic use in these areas, the emergence of different mechanisms of resistance, the selection, and spread of resistant clones under antibiotic use. Perhaps this is another reason for such studies to be a model identify the resistance of each region and hospital, and in line with that, the methods of infection control and prevention of the spread of resistance and, most importantly, help in choosing appropriate treatment methods to rid patients of infections caused by this important and highly resistant "*Escherichia coli*" pathogen (34–36).

Conclusion

ESBL and MBL resistance genes are increasing in the present study in Tehran. The presence of 2 or 3 antibiotic resistance genes in ESBL and MBL isolates increases the antibiotic resistance of bacterial isolates, which causes the phenomenon of multiple drug resistance and disrupts the treatment process. It indicates the transfer of genetic elements.

Beta-lactamases are the main defense system of gram-negative bacteria against beta-lactam antibiotics. Since beta-lactam antibiotics were used in the clinic, beta-lactamases evolved with them and played a major role in therapeutic failure in antibiotic therapy. To prevent the further prevalence of beta-lactamase genes and higher resistance to antibiotics, physicians should be trained in combination therapy and antibiogram tests before prescribing drugs, as well as antibiotics. Resistance to them is reported to be high and they should be prescribed by doctors with caution. These bacteria, like other nosocomial infections, are transmitted through the infected hands of hospital staff and contaminated medical equipment, including urinary, vascular, and arterial catheters. Due to the resistance of these bacteria to other antibiotics, today they are considered a major and growing problem in the treatment and control of infection. Therefore, infection of these bacteria can be controlled by using various strategies, including the correct use of antibiotics and limiting their use.

Beta-lactamase production is one of the most important mechanisms of resistance in bacteria. Metallo-beta-lactamases are considered as one of the most important mechanisms involved in the development of resistance to antimicrobial compounds such as carbapenems and third-generation cephalosporins.

These strains in hospitals, especially in infectious wards, can control the spread of these strains more effectively. As a result, medical staff can choose an appropriate treatment model that reduces treatment costs and serious risks, including death in this group of patients.

Declarations

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Authors' contributions

D.E., B. G. P and R.S designed this research. D.E. performed data analysis. D. E. and B.G.P. wrote the article. R.S., B. G.P, and D.E. reviewed the article.

Availability of data and materials

All data included in this article are available from the corresponding author on reasonable request.

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Competeting of Interest

No competing financial interests exist.

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Figures

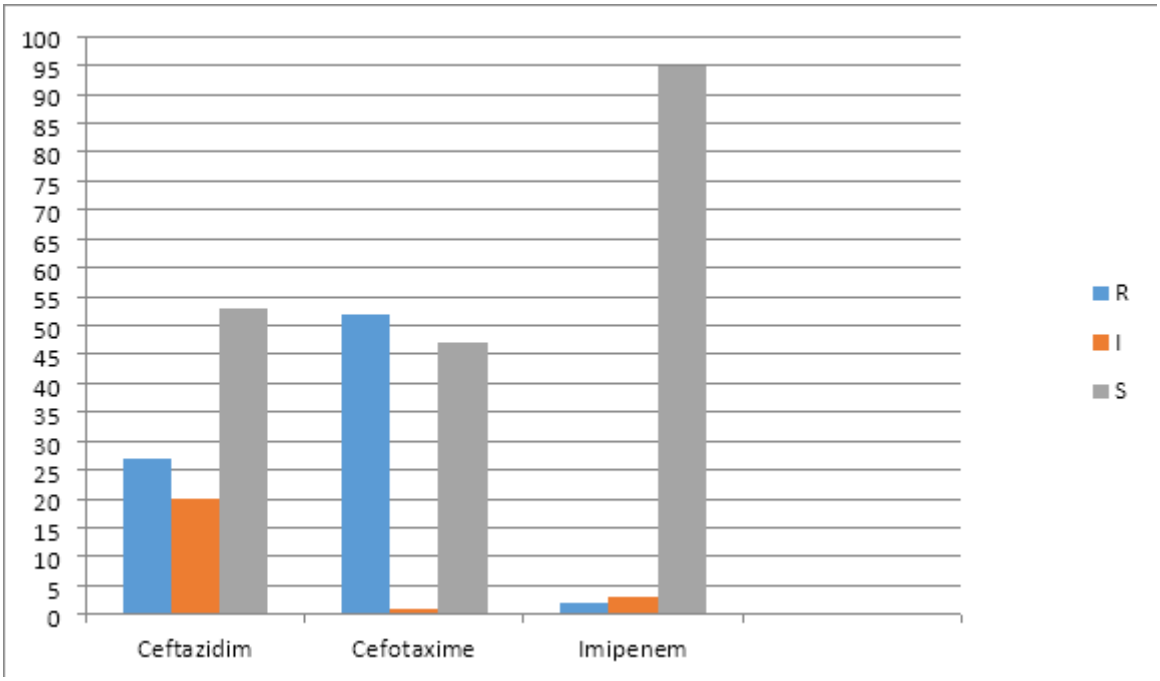

Figure 1

The distribution of antibiotic resistance among 100 clinical isolates of Escherichia coli

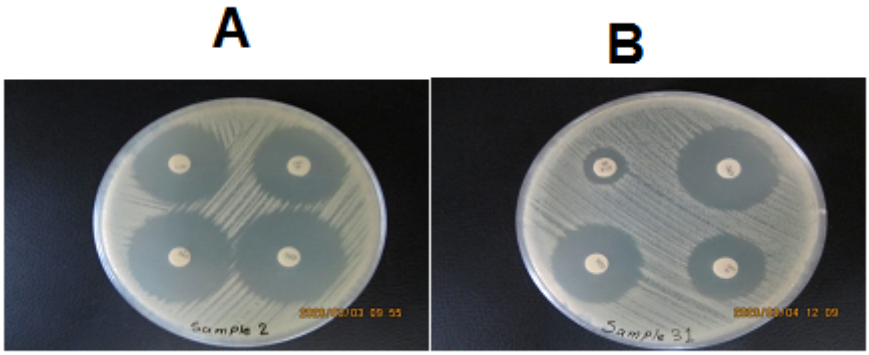

Figure 2

Results of the presence of broad-spectrum beta-lactamase: (A) bacteria without ESBL (B) bacteria with ESBL

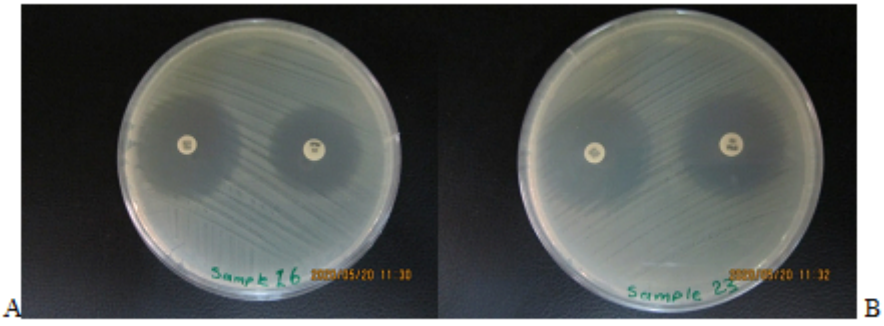

Figure 3

Results of the presence of metallo-beta-lactamase: (A) bacteria with MBL (B bacteria without MBL

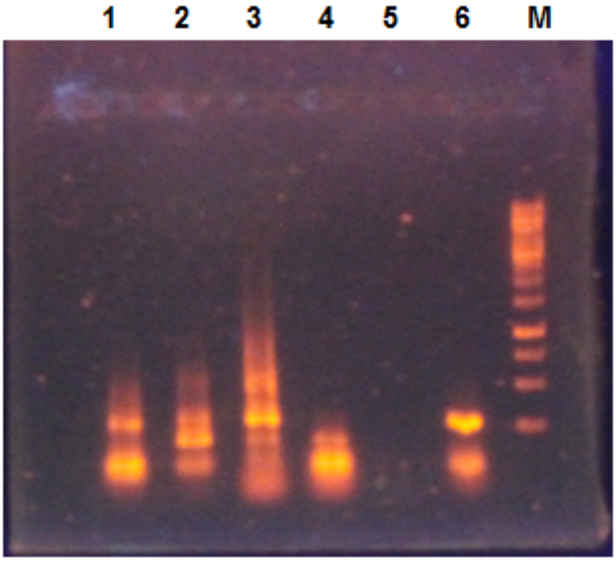

Figure 4

Electrophoresis of KPC, Ampc, TEM gene amplification products on 1% agarose gel Well M 100 bp DNA Ladder, well 1 AmpC, well 2 AmpC, TEM, well 3: AmpC, TEM, KPC, well 4: AmpC, well 5: Negative control, well 6: AmpC

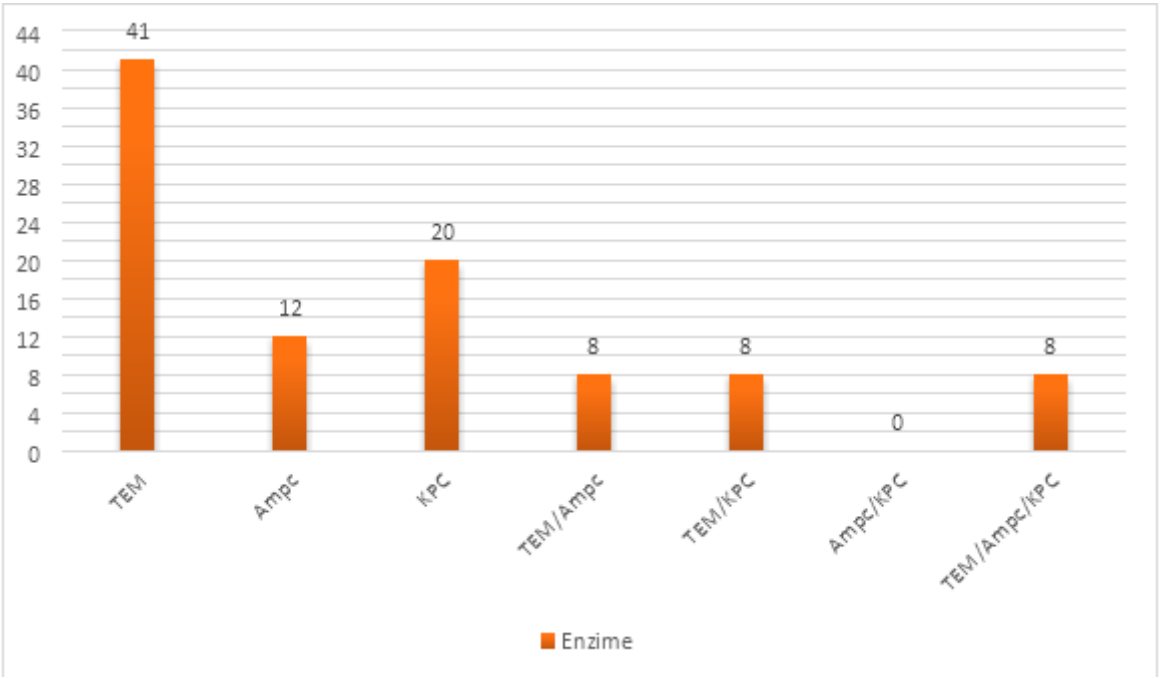

Figure 5

Results of Multiplex PCR experiments for ESBL

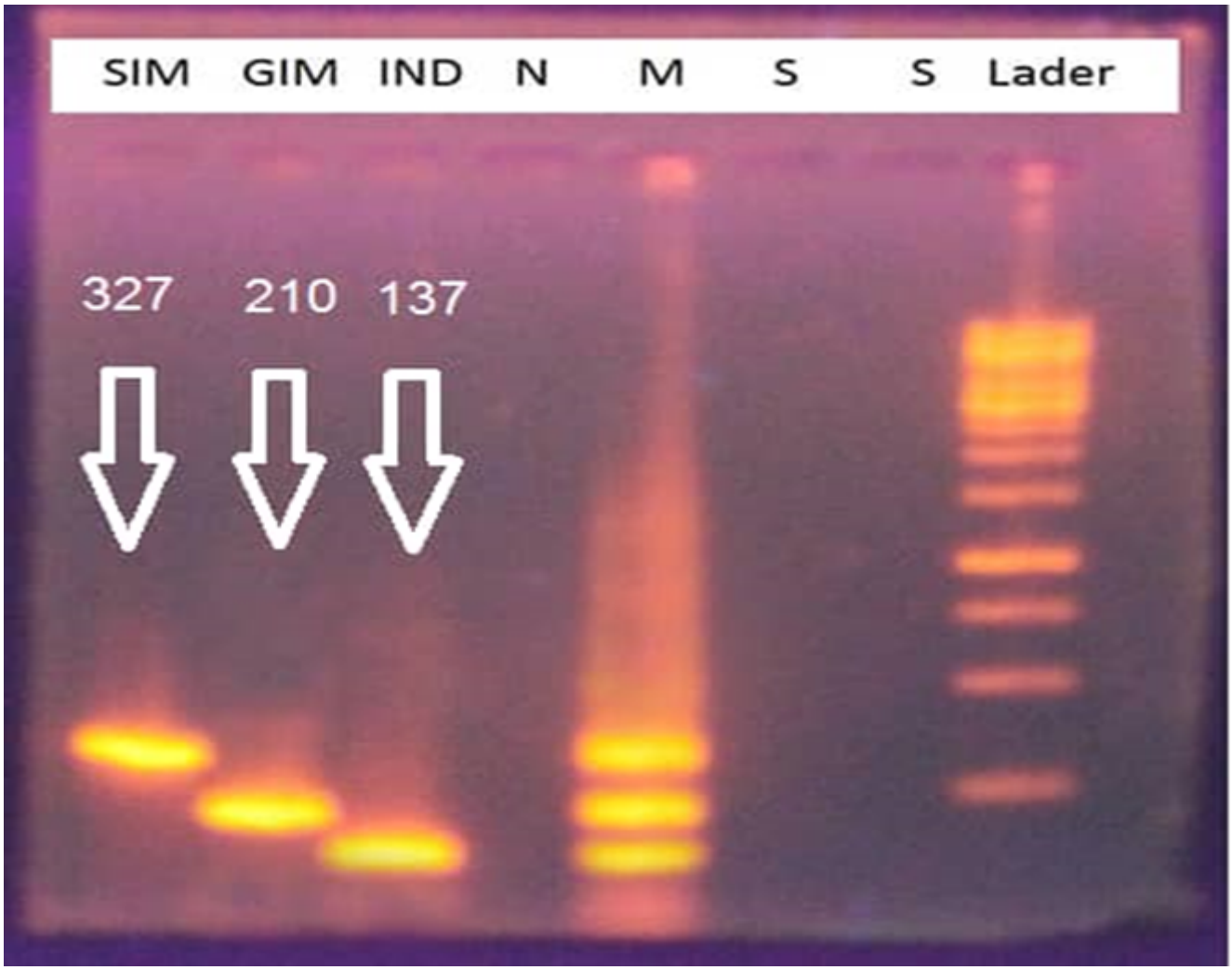

Figure 6

Electrophoresis of SIM, GIM, IND gene amplification products on 1% agarose gel well 100 bp DNA Ladder, Well S: A well containing DNA-free primers with ESBL genes Well M: The well contains primers and DNA containing all three ESBL genes, Well N: Negative Control.

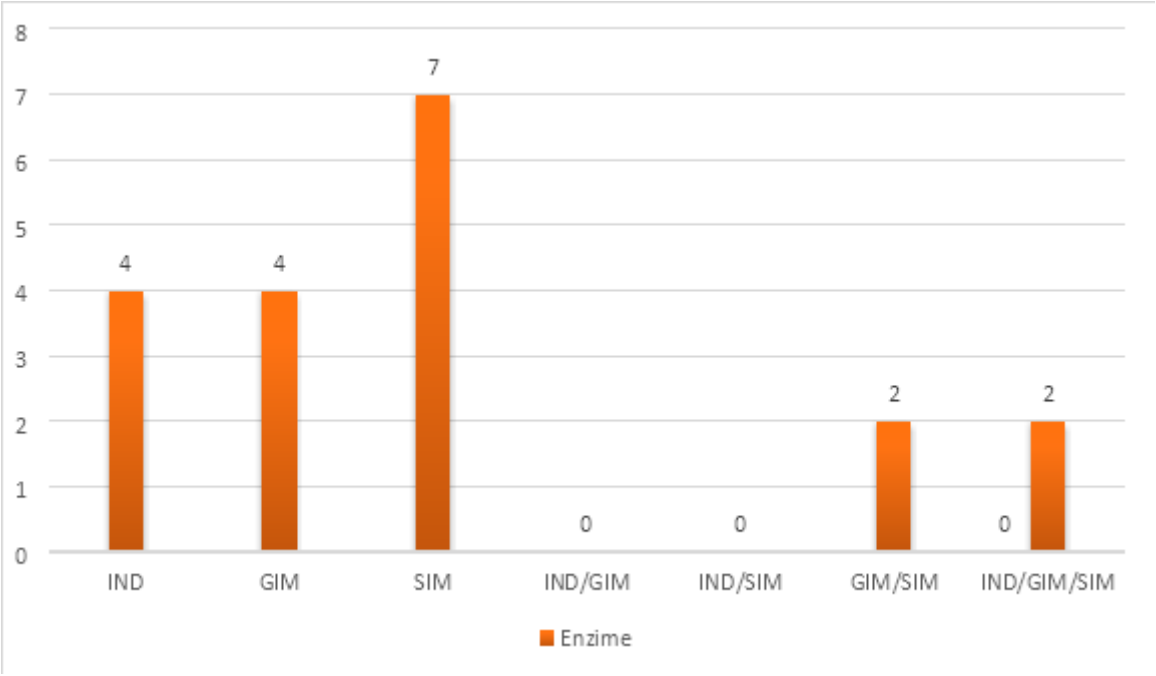

Figure 7

Results of Multiplex PCR experiments for MBL