

Research Article

TEM Gene Detection in Clinical *Pseudomonas aeruginosa* and *Escherichia coli* Samples

Elahe Shams¹, Behnaz Nateghi², Amir Eshaghiyan³, and Parisa Behshood⁴

¹Young Researchers and Elite Club, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

²Department of Biochemistry, Faculty of Science, Nourdanesh Institutions of Higher Education, Meimeh, Isfahan, Iran

³Department of Genetics, Arsanjan Branch, Islamic Azad University, Arsanjan, Shiraz, Iran

⁴Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Isfahan, Iran

Abstract

Background: Isolation of the *TEM* beta-lactamase gene from clinical *Pseudomonas aeruginosa* and *Escherichia coli* samples provides useful information on the epidemiology of and factors involved in infections caused by these agents as well as their antibiotic resistance patterns. The aim of this study was to evaluate the antibiotic resistance of *P. aeruginosa* and *E. coli* isolated from specimens obtained in Isfahan, Iran via detection of the *TEM* gene.

Materials and methods: In this cross-sectional study, 120 *P. aeruginosa* and 86 *E. coli* samples isolated from urine and sputum were identified using biochemical methods. Their antimicrobial resistance pattern was investigated using the Kirby-Bauer disc diffusion method. Then, phenotypic detection of extended-spectrum beta-lactamases (ESBL) was performed using a combined disc method. Finally, the *TEM* gene in isolated samples was examined using polymerase chain reaction (PCR).

Results: *P. aeruginosa* isolates were found to show the highest resistance to tetracycline (97.5%) and amoxicillin (95%) and the highest sensitivity to aztreonam (97.5%) and amikacin (61.66%). 68 *P. aeruginosa* samples (56.6%) contained a *TEM* gene. *E. coli* isolates were found to show the highest resistance to co-trimoxazole (59.34%) and amoxicillin (55.04%), and the highest sensitivity to imipenem (69.66%) and chloramphenicol (61.92%). 62 *E. coli* samples (72.09%) contained a *TEM* gene.

Conclusions: The alarming spread of ESBL-producing pathogens is a complicating factor in antimicrobial therapies. It is essential to employ diverse strategies for the supervision of the spread of these pathogens.

Keywords: Antimicrobial, *Escherichia coli*, *Pseudomonas aeruginosa*, *TEM*, β -lactamases

Corresponding Author:
Parisa Behshood
Young Researchers and Elite
Club, Shahrekord Branch,
Islamic Azad University,
Isfahan, Iran
Tel: +98-9131860458
email: Parisa_behshood@
yahoo.com

Production and Hosting by
Knowledge E

© Elahe Shams et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Editor-in-Chief:
Dr. Alireza Rafiei

1. Introduction

Pseudomonas aeruginosa is a major cause of infections, such as pneumonia, bacteremia, urinary tract infections, as well as cystic fibrosis, in hospitals with weak safety systems (1). *Escherichia coli* is the most common cause of urinary tract infections (2). *E. coli* strains are typically divided into four phylogenetic groups: A, B1, B2, and D

 OPEN ACCESS

(3). Uropathogenic *E. coli* (UPEC) were found to be gender-associated and in higher numbers in group B2 (2). Beta-lactam family antibiotics are used to treat infections caused by these two bacterial species. Enzymes called beta-lactamases produced by these bacteria hydrolyze the β -lactam ring of antibiotics and prevent their binding to the target site, causing antibiotic resistance (4). In *E. coli* and *P. aeruginosa* isolates, beta-lactamases were found to be encoded by plasmids (5,6). Extended-spectrum beta-lactamase (ESBL) prevalence levels between 6% and 88% were reported previously in nosocomial infections.

β -lactamases are classified into four molecular classes: A, B, C, and D. Classes A, C and D act via a serine-based mechanism, whereas class B (or MBL: metallo-beta-lactamase) β -lactamases require zinc to function. Plasmid-encoded *TEM-1* β -lactamases are the most studied class A enzymes in gram-negative bacteria. Spreading rapidly throughout the world, they are considered as the most common beta-lactam resistance mechanism in gram-negative bacilli (7,8). Observed *TEM* genes (*bla_{TEM}*) confer resistance to most antibiotics such as penicillin and first-generation cephalosporins such as cephaloridine. *TEM*-type ESBLs are derived from *TEM-1* and *TEM-2* with amino acid replacements within the active site (9). The global prevalence of *bla_{TEM}* in clinical isolates varies and has continued to change over time (10). The number of organisms producing *TEM* enzymes continues to increase, and the antibiotic resistance conferred by *TEM* enzymes remains a major crisis in the treatment of infections caused by these bacteria (11). On the contrary, *P. aeruginosa* and *E. coli* are the most important causes of hospital infections, and these pathogenic bacteria show high resistance to a wide range of antimicrobials and antibiotics. Studies on the production of beta-lactamases by these pathogens can provide a relatively adequate insight into antibiotic resistance patterns in a geographical region. Thus, the aim of this study was to determine the antibiotic resistance patterns of *P. aeruginosa* and *E. coli* using the disk diffusion method and to determine the frequency of occurrence of the *TEM* β -lactamase gene in clinical *P. aeruginosa* and *E. coli* samples using polymerase chain reaction (PCR) method.

2. Materials and Methods

2.1. Sample collection and bacterial characterization

Two hundred and twenty clinical samples (urine and sputum) were collected from clinical laboratories in Isfahan province, Iran, between February 2018 and August 2018. A hundred and twenty *P. aeruginosa* isolates and 86 *E. coli* isolates were identified according to basic biochemical tests and Laboratory Standards Institute (CLSI) guidelines. *P. aeruginosa* specimens were collected from people who were susceptible to pneumonia and displayed symptoms, such as difficulty in breathing, shortness of breath, and chest pain. *E. coli* specimens were collected from people with suspected urinary tract infections showing symptoms, such as urinary burning and frequent urination in small amounts.

2.2. Antimicrobial susceptibility test

The phenotypic detection of extended-spectrum beta-lactamases (ESBL) was performed using the double-disk diffusion (DDS) test according to clinical laboratory guidelines. Used antibiotics included chloramphenicol (30 µg), tobramycin (10 µg), co-trimoxazole (25 µg), amikacin (30 µg), ciprofloxacin (5 µg), tetracycline (5 µg), amoxicillin (30 µg), cefotaxime (30 µg), imipenem (10 µg), and aztreonam (30 µg). All discs were obtained from Hi-Media, Mumbai, India. Reference strains of *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used for the quality control of antimicrobial susceptibility tests.

2.3. DNA extraction and polymerase chain reaction

DNA of the isolates was extracted using a DNA extraction kit (Sinoclon, Iran) according to the manufacturer's protocol. The quality of the extracted DNA was measured using a spectrometer (Thermo Scientific, Waltham, MA, USA). Primer sequences for each bacterium (Table 1) were selected based on previous studies (12, 13). The PCR mixture (Sinoclon) contained 0.6 µl MgCl₂ (1.5 mM), 1 µl Taq DNA polymerase (500 U), 5 µl 10x PCR buffer, 0.4 µl dNTP (200 µM), 0.5 µl of each primer (10 pmol/ml), and 2 µl of DNA template (1 µl genomic DNA). The *bla*_{TEM} gene was amplified under the following conditions: initial denaturation at 95°C for 5 min followed by 35 cycles including denaturation at 95°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 45 s. The cycles were followed by a final extension at 72°C for 5 min. The PCR products were separated by electrophoresis in 1.5% agarose gel and visualized by staining using green viewer under UV light.

TABLE 1: Primer sequences used for PCR reaction.

Size	Target gene	Sequence	Bacteria species
867	<i>bla</i> _{TEM}	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	<i>Pseudomonas aeruginosa</i>
431	<i>bla</i> _{TEM}	AGTGCTGCCATAACCATGAGTG CTGACTCCCCGTCGTGTAGATA	<i>Escherichia coli</i>

2.4. Statistical analysis

The results are presented as mean ± standard deviation (SD) of triplicate measurements. The data were analyzed using one-way analysis of variance (ANOVA) using the SPSS software (Chicago, IL, USA).

3. Results

P. aeruginosa isolates were found to show the highest resistance to tetracycline (97.5%) and amoxicillin (95%) and the highest sensitivity to aztreonam (97.5%) and amikacin (61.66%). 68 samples (56.6%) isolated in this study contained a *TEM* gene. *E. coli* isolates

were found to show the highest resistance to co-trimoxazole (59.34%) and amoxicillin (55.04%) and the highest sensitivity to imipenem (69.66%) and chloramphenicol (61.92%). 62 samples (72.09%) contained a *TEM* gene (Figure 1 & 2).

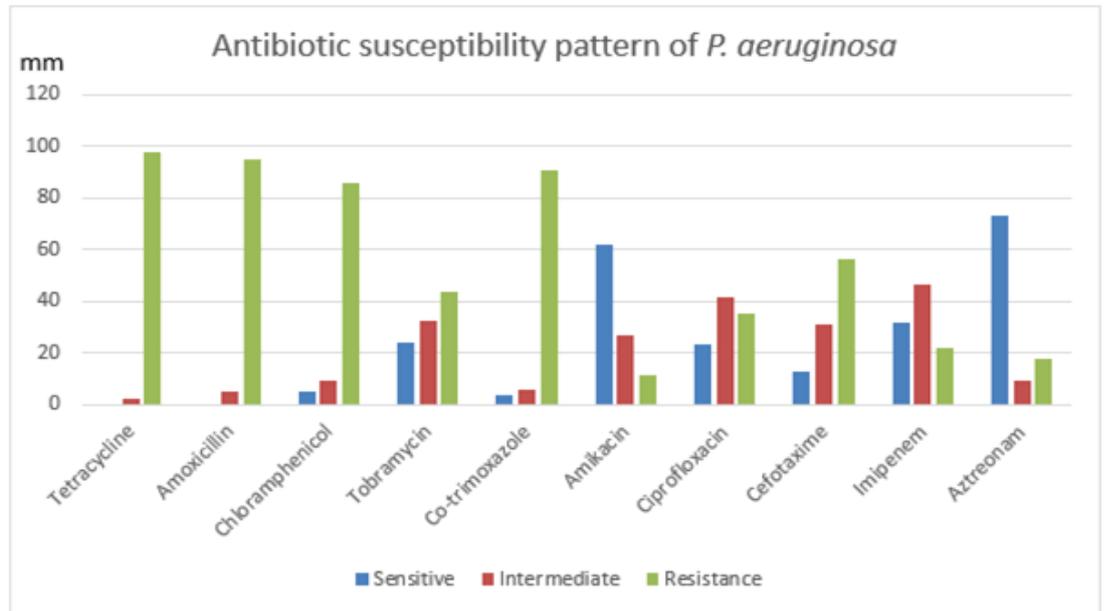


Figure 1: Antibiotic susceptibility pattern in *P. aeruginosa* isolates, inhibition zone diameters (mm).

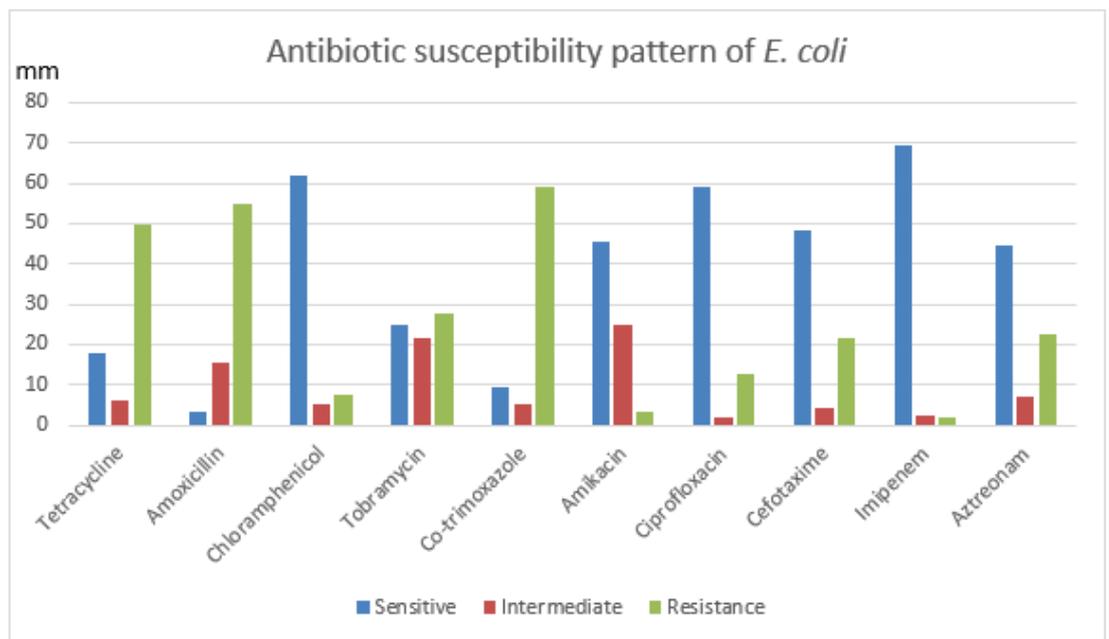


Figure 2: Antibiotic susceptibility pattern in *E. coli* isolates, inhibition zone diameters (mm).

Our findings showed that 68 (56.6 %) out of a total of 120 *P. aeruginosa* samples and 62 (72.09 %) out of a total of 86 *E. coli* samples carried the *TEM* gene, respectively (Figure 3 & 4). The samples with the *TEM* gene showed resistance to all antibiotics to varying extents.

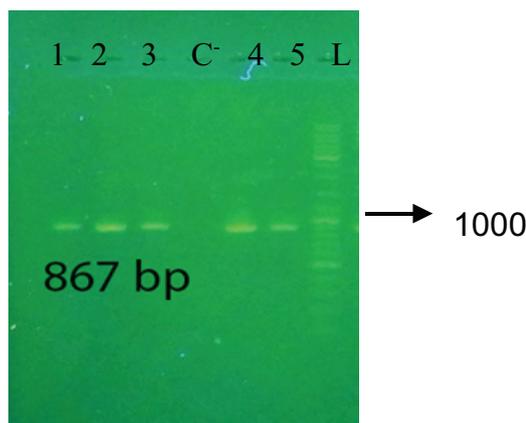


Figure 3: *TEM* amplicon in PCR product of *Pseudomonas aeruginosa* isolates (Column L: Size marker (100 bp), Column C⁻: Negative Control, Columns 1, 2, 3, 4, and 5 (867 bp): positive samples).

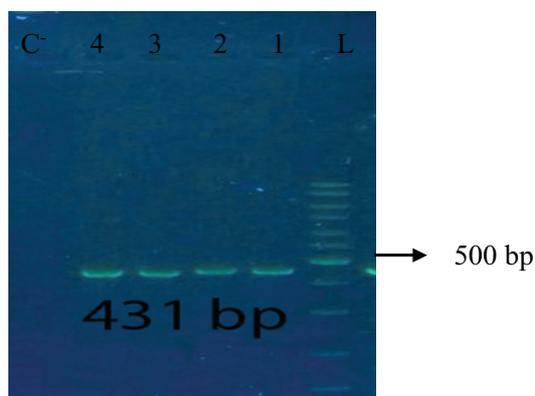


Figure 4: *TEM* amplicon in PCR product of *E. coli* isolates (Column L: Size marker (100 bp), Column C⁻: Negative Control, Columns 1, 2, 3, and 4 (431 bp): positive samples).

4. Discussion

P. aeruginosa is one of the pathogens bacteria in hospitals. Most antibiotics used against infections caused by this pathogen are currently are not very effective (14). *E. coli* is still the dominant cause of urinary tract infections worldwide, responsible for 80-90% of urinary tract infections (15). The choice of antibiotic type for the experimental treatment of urinary tract infections is still under debate, as 20-50% of the isolates are currently resistant to first-line antibiotics, even in developed countries (16). In this study, 220 urine and sputum samples were collected from laboratories in the Isfahan province. Following identity verification tests, the antimicrobial resistance patterns of samples were compared. High levels of resistance to various antibiotics, especially beta-lactams, in *P. aeruginosa* and *E. coli* were found. Most isolated strains were resistant to three or more classes of antimicrobials. Shirehjini et al. previously found high antibiotic resistance levels in *P. aeruginosa* strains and reported that 34.2% of the strains contained a *TEM* gene (17). Peymani et al. reported that the *blaTEM-1* (26.7%) in *P. aeruginosa* strains was the most frequently observed gene, followed by *blaCTX-M-15* (17.3%), *blaSHV-1* (6.7%), and *blaSHV-12* (4%) [18]. Bahrami et al. have investigated the presence of *blaSHV*, *blaTEM*, *blaCTX-M* and *blaOXA-48* beta-lactamase genes in 96 clinical isolates of *P. aeruginosa*

in Bandar Abbas by the PCR method. The prevalence of *bla*CTX-M, *bla*SHV, *bla*TEM and *bla*OXA-48 genes were 23.95% (23 isolates), 23.08% (26 isolates), 57.29% (55 isolates), and 12.5% (12 isolate), respectively (19). These findings are clearly close to those reported in this study.

Bajpai et al. determined the prevalence of ESBL (*bla*TEM, *bla*CTX-M, and *bla*SHV) genes among the members of *Enterobacteriaceae*. 78 *E. coli* and *Klebsiella* isolates were identified out of the 80 members of the *Enterobacteriaceae* isolated. The prevalence of TEM was 55.1% in *E. coli* and 58% in *Klebsiella* (20). Jena et al. have investigated the prevalence of TEM, SHV, and CTX-M genes of ESBL-producing *E. coli* strains isolated from urinary tract infections. *bla*TEM was the predominant (93.47%) gene followed by *bla*CTX-M (82.6%) and *bla*SHV (4.34%) (21). In another study, Bali et al. showed that among the ESBL-producing isolates of *E. coli*, 72.72% had the TEM gene (22). The results of these studies are similar to those reported in the present study. Toupkanlou et al. found that, of the 217 isolates, 87 were cephotaxime resistant gram-negative bacilli. 42 (48.3%) of these were found to be ESBL producers. The prevalence of *bla*SHV, *bla*TEM, and *bla*OXA-10 genes were 36% among the 50 imipenem-resistant isolates of *P. aeruginosa* (23). These frequencies are clearly close to those reported in this study. Due to the increase of ESBL genes in uropathogens, sustained supervision of the use of antibiotics and that of infection levels are essential. Information regarding the antibiotic resistance patterns of pathogens can assist physicians with the selection of suitable antibiotic regimens. Overall, in this study, the TEM gene was identified in more than half of the isolated strains. The alarming spread of ESBL-producing pathogens is a complicating factor in antimicrobial therapies. It is, thus, essential to employ diverse strategies in the supervision of the spread of these pathogens.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

P.B; Contributed to study design. A.E; Contributed to sample collection. B.N, P.B, and E.Sh; Contributed to all experimental work, molecular experiments, and statistical analysis. B.N and E.Sh; Contributed to drafted the manuscript. P.B; Contributed to discussed the findings and approved the manuscript.

References

- [1] Ullah W, Qasim M, Rahman H, Bari F, Khan S, Dworeck T, Muhammad N. Molecular identification of TEM-116 beta-lactamase gene in isolates of pathogenic *Pseudomonas aeruginosa*: A first report from Pakistan. *Trop J Pharm Res.* 2017;16(1):149-54.
- [2] Toval F, Schiller R, Meisen I, Putze J, Kouzel IU, Zhang W, Karch H, Bielaszewska M, Mormann M, Mütting J, Dobrindt U. Characterization of urinary tract infection-associated Shiga toxin-producing *Escherichia coli*. *Infect Immun.* 2014 1;82(11):4631-42.
- [3] Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol.* 2000 1;66(10):4555-8.

- [4] Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Cestodes. Medical Microbiology. 4th ed. St Louis, USA. Mosby. 2002:754-63.
- [5] Ku YH, Lee MF, Chuang YC, Yu WL. Detection of Plasmid-Mediated β -Lactamase Genes and Emergence of a Novel AmpC (CMH-1) in *Enterobacter cloacae* at a Medical Center in Southern Taiwan. J Clin Med. 2019 Jan;8(1):8.
- [6] Khosravi AD, Hoveizavi H, Mehdinejad M. Prevalence of *Klebsiella pneumoniae* encoding genes for CTX-M-1, TEM-1 and SHV-1 extended-spectrum beta lactamases (ESBL) enzymes in clinical specimens. Jundishapur J Microbiol. 2013 Dec 1;6(10).
- [7] Eiamphungporn W, Schaduangrat N, Malik A, Nantasenamat C. Tackling the antibiotic resistance caused by class A β -lactamases through the use of β -lactamase inhibitory protein. Int J Mol Sci. 2018 Aug;19(8):2222.
- [8] Seyedjavadi SS, Goudarzi M, Sabzehali F. Relation between blaTEM, blaSHV and blaCTX-M genes and acute urinary tract infections. J Acute Dis. 2016 Jan 1;5(1):71-6.
- [9] Peymani A, Naserpour-Farivar T, Zare E, Azarhoosh KH. Distribution of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing *P. aeruginosa* isolated from Qazvin and Tehran hospitals, Iran. J Prev Med Hyg. 2017 Jun;58(2):E155.
- [10] AL-Subol I, Youssef N. Prevalence of CTX-M, TEM and SHV beta-lactamases in clinical isolates of *Escherichia Coli* and *Klebsiella Pneumoniae* isolated from Aleppo university hospitals, Aleppo, Syria. Arch Clin Infect Dis. 2015; 10(2):e22540
- [11] Haghi F, Zeighami H, Keramati N, Hemmati F, Hajiahmadi F. Frequency of TEM extended spectrum beta lactamase producing *Escherichia coli* in clinical specimens by phenotypic and molecular methods in Zanjan. ZUMS Journal. 2013 1;21(85):55-63.
- [12] Komijani M, Bouzari M, Rahimi F. Detection and characterization of a novel lytic bacteriophage (vB-KpneM-Isf48) against *Klebsiella pneumoniae* isolates from infected wounds carrying antibiotic-resistance genes (TEM, SHV, and CTX-M). Iranian Red Crescent Med J. 2016 (In press).
- [13] Kim J, Jeon S, Rhie H, Lee B, Park M, Lee H, Lee J, Kim S. Rapid detection of extended spectrum β -lactamase (ESBL) for *Enterobacteriaceae* by use of a multiplex PCR-based method. Infect Chemother. 2009 1;41(3):181-4.
- [14] Nikbin VS, Abdi-Ali A, Feizabadi MM, Gharavi S. Pulsed field gel electrophoresis & plasmid profile of *Pseudomonas aeruginosa* at two hospitals in Tehran, Iran. Indian J Med Res. 2007 1;126(2):146.
- [15] Arbeloa A, Oates CV, Marchès O, Hartland EL, Frankel G. Enteropathogenic and enterohemorrhagic *Escherichia coli* type III secretion effector EspV induces radical morphological changes in eukaryotic cells. Infect Immun. 2011 1;79(3):1067-76.
- [16] Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. J Antimicrob Chemother. 2002 1;50(4):513-6.
- [17] Farzali SF, Amini K, Fatahi H. Identification of blaCTX-M, blaSHV, and blaTEM Genes in *Pseudomonas Aeruginosa* strains isolated from human and animal samples using multiplex-PCR method.
- [18] Peymani A, Naserpour-Farivar T, Zare E, Azarhoosh K. Distribution of bla(TEM), bla(SHV), and bla(CTX-M) genes among ESBL-producing *P. aeruginosa* isolated from Qazvin and Tehran hospitals, Iran. J Prev Med Hyg. 2017;58(2):E155-E60.
- [19] Bahrami M, Mohammadi-Sichani M, Karbasizadeh V. Prevalence of SHV, TEM, CTX-M and OXA-48 β -Lactamase Genes in Clinical Isolates of *Pseudomonas aeruginosa* in Bandar-Abbas, Iran. 2018.
- [20] Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med Biotechnol. 2017 Jan;7(1):12.
- [21] Jena J, Sahoo RK, Debata NK, Subudhi E. Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β -lactamase-producing *Escherichia coli* strains isolated from urinary tract infections in adults. 3 Biotech. 2017 Aug 1;7(4):244.
- [22] Bali EB, Accedil L, Sultan N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum-lactamase produced by *Escherichia coli*, *Acinobacter baumannii* and *Klebsiella* isolates in a Turkish hospital. Afr J Microbiol Res. 2010 18;4(8):650-4.
- [23] Toupkanlou SP, Peerayeh SN, Mahabadi RP. Class A and D extended-spectrum β -lactamases in imipenem resistant *Pseudomonas aeruginosa* isolated from burn patients in Iran. Jundishapur J Microbiol. 2015 ;8(8).