Reports of Biochemistry & Molecular Biology
Vol.10, No.3, Oct 2021

Original article

www.RBMB.net

Frequency of *bla*$_{CTX-M}$ and *bla*$_{TEM}$ Virulence Genes and Antibiotic Resistance Profiles among *Klebsiella pneumoniae* Isolates in Urinary Tract Infection (UTI) Samples from Hashtgerd, Iran

Shirin Sarshar[1], Reza Mirnejad[2], Ebrahim Babapour*[3]

Abstract

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is an opportunistic microorganism and one of the most important causes of urinary tract infection. This study aimed to evaluate the frequency of *K. pneumoniae* producing broad-spectrum beta-lactamase in urinary tract infection and to determine the pattern of drug resistance.

Methods: This study was performed on 50 samples of *K. pneumoniae* isolated from patients with urinary tract infection referred to the Medical Diagnostic Laboratory in Hashtgerd city. The isolates were first evaluated for antibiotic susceptibility by disk diffusion method according to the method proposed by the Clinical and Laboratory Standards Institute (CLSI). Then phenotypic detection of ESBLS was carried out by the DDST method. The frequency of gene *bla*$_{TEM}$ and *bla*$_{CTX-M}$ was determined by PCR.

Results: The highest resistance was observed to ampicillin (94%) and the highest sensitivity was observed to gentamicin (84%). 22 isolates (44%) were positive for ESBLs production. Of the 50 isolates studied, 34% had *bla*$_{CTX-M}$ and 28% had *bla*$_{TEM}$ and 11 (22%) had both genes simultaneously. Also, more than 77% of positive ESBLs isolates had the *bla*$_{CTX-M}$ gene and approximately 63.64% of positive ESBLs isolates had the *bla*$_{TEM}$ gene.

Conclusions: Given the high prevalence of antibiotic-resistant and ESBL-producing isolates, early identification of these resistant isolates and their follow-up is essential to prevent further outbreaks. It is also important to use appropriate therapeutic strategies and proper and rational administration of antibiotics by physicians.

Keywords: Antibiotic Resistance, ESBLs, *K. pneumoniae*, Urinary Tract Infection.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is an opportunistic pathogen and one of the most important bacteria involved in nosocomial infections (1, 2). The mortality rate due to this bacterium is high and causes various diseases such as urinary tract infection, septicemia, pneumonia, and intra-abdominal infections in patients hospitalized in different cities of the hospital (3). The basis of appropriate treatment in urinary tract infections caused by *K.*

1: Master of Sciences (M.Sc.) Microbiology, Department of Microbiology, Faculty of Sciences, Karaj Branch, Islamic Azad University, Karaj, Iran.
2: Professor of Medical Bacteriology, Molecular Biology Research Center, Systems biology and poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3: Assistant Professor of Microbiology, Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.
***Corresponding author: Ebrahim Babapour; Tel: +98 9121483410; E-mail: e.babapour@kiau.ac.ir.**
Received: 7 Feb, 2021; Accepted: 25 Apr, 2021

[DOI: 10.52547/rbmb.10.3.412]

pneumoniae is the selection of a high-performance and not expensive antibiotic but the main problem in the treatment of these infections is the resistance of bacteria to a large number of common antibiotics (4). Antibiotic resistance in these bacteria has caused major problems in the treatment of infected patients (4). Increasing the use of antibiotics, especially third-generation cephalosporin, has led to the spread of extended-spectrum beta-Lactamase (ESBLs) producing bacteria (5). Beta-lactamases prevent the antimicrobial effect of the drug on the synthesis of bacteria walls by destroying the beta-lactam ring in drugs (6). Cefotaximases (CTX-M-ases) enzymes and beta-lactamase (TEM) are the most widespread beta-lactamases in *Escherichia coli (E. coli)* and *K. pneumoniae* that their encoded enzymes confer resistance against treatments. Currently, the TEM enzyme is considered one of the most important resistance mechanisms against beta-lactam antibiotics among gram-negative bacteria. Cefotaximases (CTX-M-ases) enzymes are a new family of broad-spectrum plasmid beta-lactamases produced by different genera of the Enterobacteriaceae family, including *K. pneumoniae*. These enzymes cause resistance to penicillin and a wide spectrum of third generation cephalosporins such as ceftazidime ceftriaxone, cefotaxime, and monobactams such as aztreonam. Extended-spectrum β-lactamase (ESBL) genes are located on large plasmids (more than 100 Kbp), which at the same time carry resistance genes to other antimicrobial agents such as aminoglycosides, chloramphenicol, sulfonamides, and tetracycline (6). Also, the information provided by many articles indicates the key role of these genes in resistance to many antibiotics used in the treatment of urinary tract infection (7). Determination of antibiotic resistance patterns in common pathogenic bacteria is important in treatment of infections (5). Accordingly, this study aimed to isolate *K. pneumoniae* from patients with urinary tract infection, investigate antibiotic resistance, screening of ESBL-producing isolates by the phenotypic method. In addition, the frequency of genes encoding beta-lactamase enzymes (TEM and CTX-M) in *K. pneumoniae* isolates producing these enzymes by Polymerase chain reaction (PCR) were investigated.

Material and methods

Samples collecting and detecting bacteria

This cross-sectional study was performed on 50 *K. pneumoniae* isolated from patients with urinary tract infection referred to a medical diagnostic laboratory in Hashtgerd, Iran, in 2019-2020. Specific biochemical tests used to differentiate species based on fermentation characteristics of sugars by bacteria (in TSI media), indole production, MR and VP test results, movement in SIM, urease, oxidase test, and lysine decarboxylase test.

Determination of antibiotic susceptibility by disk diffusion method

The Kirby-Bauer disk-diffusion method was performed regards to the recommendation of the Clinical & Laboratory Standards Institute (CLSI) 2019 (8). The sensitivity levels for ampicillin (10 µg), gentamicin (10 µg), ceftazidime (3 µg), cefotaxime (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg) (Mast Group Ltd UK), were considered. The results were classified as susceptible, intermediate, and resistant. In this experiment, *E. coli* ATCC 25922 was used as positive control.

Identification of ESBLs producing bacteria by combined disk method

To identify bacteria producing ESBLs, the screening test was performed according to CLSI (2019) guidelines using ceftazidime and cefotaxime antibiotic disks (Mast Group Ltd UK) (8). To do this, the inhibition zone of each antibiotic was measured for all bacteria. The combination disk method was used in the confirmatory test. For this purpose, ceftazidime-clavulanic acid (30/10 µg) and cefuroxime-clavulanic acid (30/10 µg) were used by the Disk-diffusion method. E. coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as negative and positive control, respectively.

[DOI: 10.52547/rbmb.10.3.412]

Polymerase chain reaction (PCR)

Total DNA of bacteria was extracted by boiling method and then PCR was performed for the detection of beta-lactamase, *bla*$_{TEM}$, and *bla*$_{CTX-M}$ genes (9). The reaction mixture consists of 12.5 µl of 2x PCR Master Mix purchased from Amplicon Company (Tris-HCl 0.5 M, MgCl2 2 mM, dNTPs 1.6 mM, Taq 0.04 Units/µl, 0.5 µl of Forward primer and 0.5 µl of each Reverse Primer (0.2 µM) were used for each gene plus 2 µl of DNA (20 ng), and 9.5 µl of sterilized distilled water.

Each PCR program consisted of 30 cycles under the following conditions:

Initial denaturation at 94 °C for 10 minutes, 30 thermal cycles including denaturation at 94 °C for 60 seconds, annealing (60 °C for *bla*$_{TEM}$ and 61°C for *bla*$_{CTX-M}$), extension at 72 °C for 60 seconds, and a final extension at 72 °C for 10 minutes. Then, PCR products were evaluated by electrophoresis on agarose gel 1% and after staining with SAFE Stain DNA (CinnaGen co. Iran). Deionized distilled water was used as a negative control and the DNA of *K. pneumoniae* ATCC 700603 strain was used as a positive control.

Table 1. Oligonucleotide sequence of initiators used in this study.

Genes	Primers (5´-3´)	Tm °C	Product size	Reference
bla $_{TEM}$	F: GCTCACCCAGAAACGCTGGT R: CCATCTGGCC CCAGTGCTGC	60	*686*	7
bla $_{CTX-M}$	F: ACCGCCGATAATTCGCAGAT R: GATATCGTTGGTGGTGCCATAA	61	585	8

Statistical analysis

The Spearman correlation coefficient and Chi-square test and $p \leq 0.05$ were calculated using Microsoft Excel 2010 software and SPSS software (2019).

Results

Antibiogram tests

The results of the antibiogram test by the Kirby-Bauer method were shown on 50 isolates of *K. pneumoniae* in Table 2. The resistance to ampicillin (94%), ceftazidime and cefotaxime (44%), nitrofurantoin (34%), ciprofloxacin (30%). and gentamicin (10%) was determined. The highest sensitivity was found for gentamicin (84%) and ciprofloxacin (70%) (Fig. 1).

Table 2. Antibiotic Susceptibility Test Results of Isolated Isolates.

Antibiotics	µg/disk	Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicillin	10	94	6	0
Gentamicin	10	10	6	84
Ceftazidime	30	44	0	56
Cefotaxime	30	44	0	56
Nitrofurantoin	300	34	12	56
Ciprofloxacin	5	30	0	70

[DOI: 10.52547/rbmb.10.3.412]

Double disk Synergy Test (DDST)

Positive ESBLs isolates were confirmed according to CLSI guidelines using Double Disk Synergy Test (DDST). All 22 isolates resistant to ceftazidime and cefotaxime were confirmed as ESBL positive.

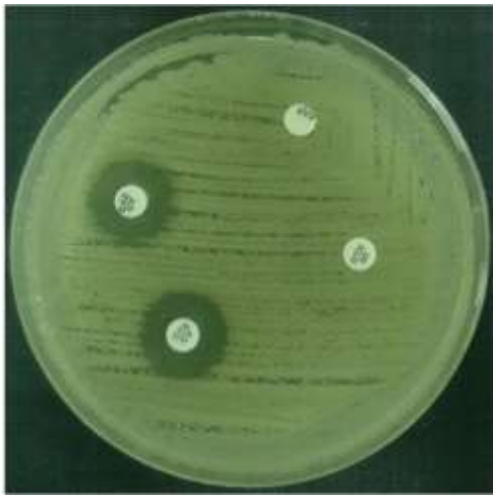

Fig. 1. Evaluation of broad-spectrum beta-lactamase-producing bacteria using disks of ceftazidime (30 µg) and ceftazidime clavulanic acid (30/10 µg) and cefotaxime (30 µg) and cefotaxime-clavulanic acid (30/10 µg).

Frequency of bla_{CTX-M} and bla_{TEM} genes using PCR

All 50 isolates were subjected to PCR and the results showed that 34% of isolates were bla_{CTX-M} positive and 28% contained bla_{TEM} (Fig. 2). The analysis showed that out of 22 isolates that were phenotypically producing beta-lactamase enzymes, 11 isolates had both bla_{CTX-M} and bla_{TEM} genes, simultaneously (Fig. 3). All isolates with bla_{CTX-M} and bla_{TEM} genes were phenotypically ESBL positive and these genes were not observed in any of the ESBL negative isolates. All 17 and 14 isolates that had bla_{CTX-M} and bla_{TEM} genes, respectively, were also ESBL positive, and out of 22 ESBL positive isolates, 20 isolates (90.9%) had at least one of the two genes, 11 isolates, both genes (50%) and only two ESBL positive isolates (9.1%) lacked both studied genes.

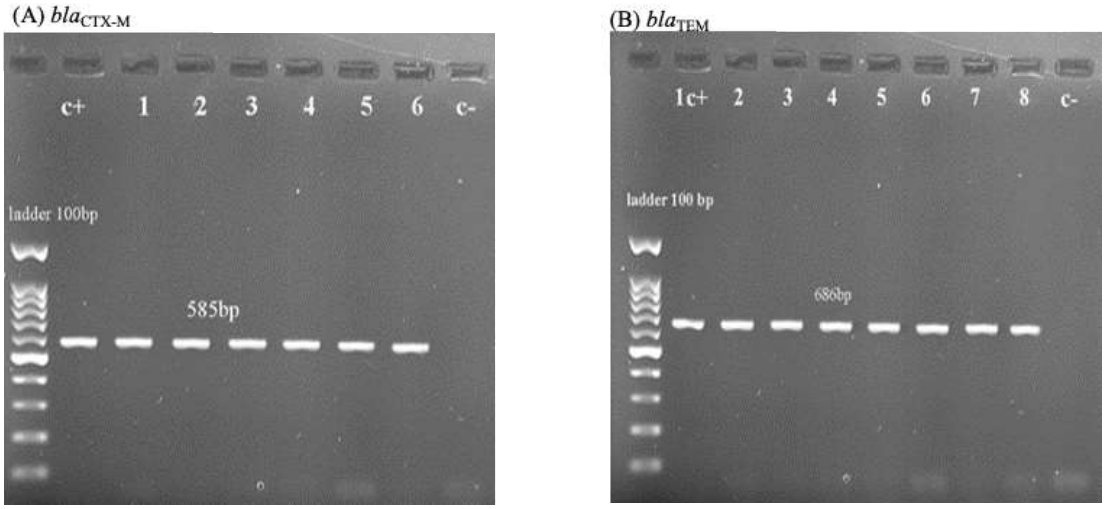

Fig. 2. bla_{CTX-M} and bla_{TEM} genes electrophoresis. (A) bla_{CTX-M}, first-row ladder well 100 bp, second-row positive control well (C +), wells 1 to 6 are positive samples and last row well as the negative control (C-). (B) $bla_{TEM,}$ first-row ladder well 100 bp, second-row positive control well (C +), wells 2 to 8 samples positive and last row well negative control (C).

[DOI: 10.52547/rbmb.10.3.412]

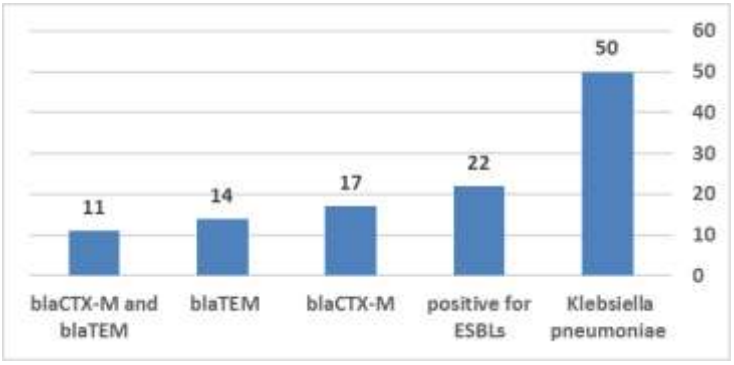

Fig. 3. Number of isolates, frequency of positive ESBLs isolates, isolates with the $bla_{\text{CTX-M}}$ gene, isolates with bla_{TEM} and isolates with both genes, $bla_{\text{CTX-M,}}$ and bla_{TEM}.

Discussion

The resistance frequency to ampicillin antibiotic we found here was similar to the study conducted by Abdolahi et al. They reported 88% resistance to ampicillin antibiotic (10). In Mansouri et al study, the highest amoxicillin resistance (100%) was detected (11). The susceptibility to gentamicin in this study was 84%, which is consistent with the results by Mohajeri et al, which was 85% (12). Pakzad et al, found lower sensitivity to gentamicin (73%) (13). Derakhshan et al and Feizabadi et al found the rate of gentamicin antibiotic resistance in Tehran was 60% and 34.8%, respectively (14, 15). The level of resistance in this study is very high compared to our study, which can be due to the time of the study, the type of samples, and the number of samples studied. In the present study, 30% of the isolates were resistant to ciprofloxacin. Ciprofloxacin resistance was reported to be 75% in the studies of Cao et al, Goudarzi et al, 60.8%, and Jacoby et al, 50% (16-18) which was more than the present study. However, it is consistent with a study conducted by Peymani et al in 2015, in which 34.5% of ciprofloxacin-resistant isolates of *K. pneumoniae* were stated (19). Also, the results of the present study differed from a study conducted by Shams et al in 2015, in which high resistance to ciprofloxacin was reported among *K. pneumoniae* isolates (20). The results of resistance to ceftazidime and cefotaxime antibiotics in this study with the results of Sedaghatpishe et al who stated resistance to these two antibiotics 54% and 36% respectively and with Asgari et al study in which 24% of *K. pneumoniae* isolates to two antibiotics ceftazidime and cefotaxime were identified to be slightly different (21,22). However, there are several reasons for the variability of drug susceptibility outcomes of a bacterium in different studies, including study time, differences in geographical logic and origin of samples, the number of isolates studied, as well as the type of disks studied, and the sensitivity of the method studied (23). Another aim of this study was to evaluate the presence of beta-lactamase genes in *K. pneumoniae* isolates obtained from human urinary tract infections. In the present study, for initial screening, the isolates were first evaluated for resistance to ceftazidime and cefotaxime antibiotics, and then the DDST phenotypic test was performed for confirmatory testing to identify positive ESBLS isolates. Based on the results; 22 isolates (44%) were positive for ESBLs. In the study of Mansury et al, it was shown that 26.3% of the 144 *K. pneumoniae* isolated from different samples are ESBLs, which is almost different from our study (24). However, the results of this study are consistent with the results of Nematzadeh et al, who stated that 40.8% of *K. pneumoniae* isolates studied by ESBLs are positive (25). Also, the rate of ESBLs-producing isolates in the studies of Asgari et al 25%, Shahcheraghi et al, 33%, Nasehi et al, 38.5% and in the study of Feizabadi et al, 69.7% have been reported (15, 22, 24, 26); some of these results are

[DOI: 10.52547/rbmb.10.3.412]

consistent with the results of the present study and some of the results are more and less than the present study. The reason for the difference in results can be more related to the number of samples studied and the time and differences in geographical logic and the origin of the samples.

In this study, the PCR method was used to identify bla_{TEM} and $bla_{CTX\text{-}M}$ genes. The obtained results showed that; Of the 50 isolates studied, 34% had the $bla_{CTX\text{-}M}$ gene and 28% had the bla_{TEM} gene and 11 isolates (22%) had both genes at the same time. Of the 22 isolates in which ESBLs were positive, 20 isolates (90.9%) had at least one of the two genes studied and 11 isolates (50%) had both genes; Only 2 isolates of positive ESBLs (9.1%) did not have both genes studied and also more than 77% of isolates of positive ESBLs had the $bla_{CTX\text{-}M}$ gene and approximately 63.64% of the isolates had ESBLs were positive for bla_{TEM} gene. Statistical analysis did not show a significant relationship between these isolates in terms of resistance to the studied antibiotics and the presence of the studied genes. But studies have shown that ESBL was positive in all bacteria containing these genes. However, the two ESBL-positive bacteria did not have any of the two genes studied, which could indicate the role of other genes in resistance to beta-lactam antibiotics. The information provided by many articles indicates the key role of these genes in resistance to many of the antibiotics used to treat urinary tract infections (22-28). The frequency of the mentioned genes in different studies in Iran is very different and these differences can be due to the clonal release of organisms that produce these enzymes in different regions or hospitals or even wards (21). Comparison of the results of the present study with the study of other researchers has similarities and differences, including the study of Shahcheraghi et al in 2007 in Tehran showed that 32% of *K. pneumoniae* isolates are resistant to cefotaxime and 31% to ceftazidime and the prevalence of bla_{SHV} and bla_{TEM} to the equations were 69.6% and 32.1%, respectively, which is almost close to the results of this study (29). In the study of Mansouri et al, the prevalence of $bla_{CTX\text{-}M}$ and bla_{TEM} beta-lactamase genes was 19% and 16%, respectively, which was lower than the present study (11). Also, the study of Asgari et al showed that 20 isolates out of 25 isolates of positive ESBLs (80%) contained the bla_{TEM} gene and 22 isolates (88%) contained bla_{SHV} gene and 68% contained both genes (22). In the study of Nasehi et al, bla_{TEM} was observed in 26% of Klebsiella isolates (26). In the study of Nematzadeh et al, in 2011 in Tehran hospitals, it was shown that out of 250 isolates of *K. pneumoniae* isolates, 102 isolates were ESBLs, which is completely consistent with the results of this study and with molecular analysis of the $bla_{CTX\text{-}M}$ gene (71.5%). In particular, CTXM-1 and CTXM-3 had 35.61% and 21.9% frequency, respectively. According to their results, the $bla_{CTX\text{-}M}$ gene is the most abundant among isolated Klebsiella isolates; which was similar to the results of the present study (25).

The above results show that the indiscriminate use of antibiotics has led to bacterial resistance to antibiotics and the increasing prevalence of ESBL-producing bacteria. ESBL-producing *K. pneumoniae* prevalent in society, it seems that screening for infections caused by ESBL production will lead to a more effective antibiotic treatment. The results also showed that genes $bla_{CTX\text{-}M}$ and bla_{TEM} in the prevalence of ESBLs bacteria have a key role and a $bla_{CTX\text{-}M}$ gene more quickly than other beta-lactamase-producing genes, is increasing; therefore, it is suggested that by changing the strategy of antibiotic use, suitable conditions be provided in different wards of the hospital and, as far as possible, the transmission of these resistances and the spread of related genes be prevented.

Acknowledgements

This manuscript is from master's microbiology graduate thesis. The authors of this article would like to express their gratitude to the dean of the Faculty of Science and the experts of the Microbiology Research Laboratory of the Islamic Azad University, Karaj Branch.

[DOI: 10.52547/rbmb.10.3.412]

References

1. Chanbari M, Mirnejad R, Babapour E. Evaluation of Resistance to Fluoroquinolones and Its Relationship whit parC Gene Mutation in *Klebsiella pneumoniae* Clinical Isolates. Iranian Journal of Medical Microbiology. 2020;14(3):270-289.
2. Razavi S, Mirnejad R, Babapour E. Involvement of AcrAB and OqxAB Efflux Pumps in Antimicrobial Resistance of Clinical Isolates of *Klebsiella pneumonia*. Journal of Applied Biotechnology Reports. 2020;7(4): 251-257.
3. Mahmood A, Al Hakawati MI. Non-beta-lactam Antimicrobials versus Extended Spectrum Beta-lactamase Producing Gram-Negative Bacteria: *In vitro* Susceptibility Study. Infect Dis Soc of Pak. 2011;43(5):507-11.
4. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998;11(4):589-603.
5. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended-spectrum beta-lactamase in urinary isolates. Indian J Med Res. 2004;120(6):553-6.
6. Kalaskar A, Venkataramana K. Determination of antimicrobial resistance pattern and production of extended-spectrum B-lactamases amongst *Escherichia coli* and *Klebsiella pneumoniae* from clinical isolates. Journal of Medical Bacteriology. 2015;1(3-4):17-24.
7. Liang C, Xing B, Yang X, Fu Y, Feng Y, Zhang Y. Molecular epidemiology of aminoglycosides resistance on *Klebsiella pneumonia* in a hospital in China. Int J Clin Exp Med. 2015;8(1):1381-5.
8. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Twenty-nine Informational Supplement. Wayne: Clinical and Laboratory Standards Institute; 2019. Available from: http://ncipd.org/control/images/NCIPD_docs/CLSI_M100-S24.pdf.
9. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2006;27(4):351-3.
10. Abdolahi AR, Mehrazma M. Evaluation of antibiotic susceptibility and resistance in urinary infection, Imam Khomeini hospital, Complex-Tehran. Journal of Jahrom University of medical sciences. 2009;7(2):59-66.
11. Mansouri S, Neyestanaki DK, Shokoohi M, Halimi S, Beigverdi R, Rezagholezadeh F, et al. Characterization of AmpC, CTX-M and MBLs types of β-lactamases in clinical isolates of *Klebsiella pneumoniae* and Escherichia coli producing Extended Spectrum β-lactamases in Kerman, Iran. Jundishapur J Microbiol. 2014;7(2):e8756.
12. Mohajeri P, Izadi B, Naghshi N. Antibiotic sensitivity of *Escherichia coli* isolated from urinary tract infection referred to Kermanshah central laboratory. JOURNAL OF KERMANSHAH UNIVERSITY OF MEDICAL SCIENCES. 2011;15(1):e79392
13. Pakzad I, Karin MZ, Taherikalani M, Boustanshenas M, Lari AR. Contribution of AcrAB efflux pump to ciprofloxacin resistance in *Klebsiella pneumoniae* isolated from burn patients. GMS Hyg Infect Control. 2013;8(2):15.
14. Derakhshan S, Najar Peerayeh S, Fallah F, Bakhshi B, Rahbar M, Mohammad-Zadeh M. Identification of Extended Spectrum Beta-lactamase producing *Klebsiella pneumoniae* isolated from Intensive Care Unit (ICU) patients in three hospitals in Tehran. Infection, Epidemiology, and Medicine. 2013;1(1):9-13.
15. Feizabadi MM, Delfani S, Raji N, Majnooni A, Aligholi M, Shahcheraghi F, et al. Distribution of *bla TEM*, *bla* $_{SHV}$, *bla* $_{CTX-M}$ Genes Among Clinical Isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. Microbial drug resistance. 2010;16(1):49-53.
16. Cao X, Cavaco LM, Lv Y, Li Y, Zheng B, Wang P, et al. Molecular characterization and antimicrobial susceptibility testing of Escherichia coli isolates from patients with urinary tract infections in 20 Chinese hospitals. J Clin Microbiol. 2011;49(7):2496-501.
17. Goudarzi M, Azad M, Seyedjavadi SS. Prevalence of Plasmid- Mediated Quinolone

[DOI: 10.52547/rbmb.10.3.412]

18. Resistance Determinants and OqxAB Efflux Pumps among Extended-Spectrum-Lactamase Producing *Klebsiella pneumoniae* Isolated from Patients with Nosocomial Urinary Tract Infection in Tehran, Iran. Scientifica (Cairo). 2015;2015:518167.
19. Jacoby GA, Chow N, Waites KB. Prevalence of plasmid-mediated quinolone resistance. Antimicrob Agents Chemother. 2003;47(2):559-562.
20. Peymani A, Farivar TN, Nikooei L, Najafipour R, Javadi A, Pahlevan AA. Emergence of plasmid-mediated quinolone-resistant determinants in *Klebsiella pneumoniae* isolates from Tehran and Qazvin provinces, Iran. J Prev Med Hyg. 2015;56(2):E61-E65.
21. Shams E, Firoozeh F, Moniri R, Zibaei M. Prevalence of plasmid-mediated quinolone resistance genes among extended-spectrum β-Lactamase-producing *Klebsiella pneumoniae* human isolates in Iran. J Pathog. 2015;2015:434391.
22. Sedaghatpishe S, Ghane M, Babaeekhou L. Identification and sequencing of *bla* CTX-M genes in clinical isolates of *Klebsiella pneumoniae* from Milad hospital, Birjand University of Medical Sciences. 2020;26(4):315-326.
23. 22. Asgari S, Haddadi A, Harzandi N. Frequency of TEM and SHV in the extended-spectrum beta-lactamase-producing Klebsiella isolates from Karaj city. Medical Sciences. 2015;25(4):277-282.
23. Wintermans B, Reuland E, Wintermans R, Bergmans A, Kluytmans J. The cost-effectiveness of ESBL detection: towards molecular detection methods?. Clin Microbiol Infect. 2013;19(7):662-5.
24. Mansury D, Motamedifar M, Sarvari J, Shirazi B, Khaledi A. Antibiotic susceptibility pattern and identification of extended-spectrum β-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* from Shiraz, Iran. Iran J Microbiol. 2016;8(1):55-61.
25. Nematzadeh S, Shahcheraghi F, Feizabadi MM, Nikbin VS, Nasehi L. Molecular characterization of CTX-Mβ-lactamases among *Klebsiella pneumoniae* isolated from patients at Tehran hospitals. Indian J Med Microbiol. 2011;29(3):254-257.
26. Shahcheraghi F, Moezi H, Feizabadi MM. Distribution of TEM and SHV beta-lactamase genes among *Klebsiella pneumoniae* strains isolated from patients in Tehran. Med Sci Monit. 2007;13(11):BR247-250.
27. Nasehi L, Shahcheraghi F, Nikbin V, Nematzadeh S. PER, CTX-M, TEM and SHV Beta-lactamases in Clinical Isolates of *Klebsiella pneumoniae* Isolated from Tehran, Iran. Iranian Journal of Basic Medical Sciences, 2010;13(3): 111-118.
28. Mirzaee M, Owlia P, Mansouri S. Distribution of CTX-M β-lactamase genes among *Escherichia coli* strains isolated from patients in Iran. Science. 2009;40(12):724-7.

[DOI: 10.52547/rbmb.10.3.412]