JCOMS

http://journalofcoms.com

Journal of

Current Oncology and Medical Sciences

Vol. 1, No.2

Original Free Access

Prevalence of *CTX-M*, *OXA* and *KPC* genes in *Klebsiella pneumoniae* isolates obtained from patients

Majid Alipour [1]*, Ghoncheh Kashani[1]

[1] Department of Cell and Molecular Biology, Babol Branch, Islamic Azad University, Babol, Iran

Abstract

Introduction: *Klebsiella pneumoniae* is known as one of the most important factors in the development of opportunistic infections. The main problem in the treatment of infections caused by these organisms is the emergence of strains with multiple resistance, which often leads to prolonged hospital stays, increased mortality and mobility, increased treatment costs compared to antibiotic-sensitive microbes, and ultimately treatment failure. Therefore, the aim of this study was to investigate the prevalence of *CTX-M*, *OXA* and *KPC* genes in *Klebsiella pneumoniae* isolates obtained from patients.

Materials and Methods: In this study, 63 isolates of *Klebsiella pneumoniae* were obtained from different clinical specimens. After final diagnosis of the strains using standard biochemical and microbiological methods, cellular DNA was obtained using Cinaclon's DNA extraction kit. Finally, multiplex-PCR test was performed to evaluate the presence of *OXA*-48, *CTX-M* and *KPC* genes in Eppendorf device using a pair of specific primers.

Results: Out of 63 samples under study, 29 samples (46%) from urine, 15 samples (23.8%) from sputum, 9 samples (41.3%) from fecal samples, 5 samples (7.9%) from wound culture and 4 samples (6.3%) were obtained from intravascular catheter of blood culture and 1 (1.6%) sample was obtained from cerebrospinal fluid. The results of PCR test for the studied genes showed that 49 (77.8%), 49 (77.8%) and 46 (73%) strains carried *OXA*, *KPC* and *CTX-M* genes, respectively.

Conclusion: The results of this study indicate that the frequency of resistance genes in *Klebsiella pneumoniae* strain is high and these strains can transfer resistance genes with high potential to other strains. Therefore, detection of *Klebsiella pneumoniae* strains containing beta-lactamase resistance enzymes is important for better treatment and prevention of the spread of these genes to other bacteria using accurate phenotypic and genotypic methods.

Keywords: *Klebsiella pneumoniae*, Antibiotic resistance, PCR

*Corresponding Author: Majid Alipour

✉ Email: alipourmk@gmail.com

Received: 2021.10.27, Accepted: 2021.11.30

Introduction

Klebsiella organisms are immobile and usually encapsulated. They ferment some sugars such as lactose and sucrose. Most strains produce gas from sugars, and the production of gas from starch is an important diagnostic feature. Almost all grow in citrate and Moller KCN environments. These bacteria are found in the intestines, stomachs and respiratory tract of humans and animals. Their G + C content is 52-58% and *Klebsiella pneumoniae* is an indicator of this group of bacteria. This bacterium belongs to the Enterobacteriaceae family (1). Depending on the type of polysaccharide in the capsule, there are three capsule serological types A, B, C in *Klebsiella pneumoniae* . The other three types D, E, F among *Klebsiella pneumoniae* under the species Ozone were also added to this collection and in 1949 it reached more than 8 types and in the following years, it was renamed to letters 1, 2, 3, etc. In the structure of these bacteria, there is O antigen, which has several different types (2). *Klebsiella pneumoniae* is an important cause of community-acquired and hospital-acquired infections. This bacterium is one of the most common nosocomial pathogens that cause high mortality and causes various types of infections, especially in infants, including pneumonia, sepsis, diarrhea, liver abscess, endophthalmitis, meningitis, urinary tract infections and bacteremia. Turns. Four million babies die every year. The highest mortality rates in infants are related to pneumonia, sepsis, meningitis and diarrhea. Infants are more vulnerable due to a lack of a complete immune system. Treatment of infections in infants infected with multidrug-resistant organisms has become a serious problem (3). *Klebsiella pneumoniae* can cause infections of the respiratory tract, nasal mucosa, pharynx and pneumonia. Pneumonia is one of the major nosocomial infections (33 to 8%). *Klebsiella pneumoniae* is the second most common bacterial infection after Escherichia coli (4). It is commonly found in people with weakened immune systems, such as hospitalized patients, diabetics, and those with chronic lung disease. Alcoholics are often exposed to the bacterium. Therefore, this infection comes from both the community and the hospital. Studies in different communities show that most of the etiological causes of urinary tract infections are bacteria of the Enterobacteriaceae family, among which Klebsiella is the cause of 16-17% of urinary tract infections. Urinary tract infection is one of the most common infections in all age groups that failure to diagnose and treat in time can cause serious complications such as urinary disorders, hypertension, renal disorders, uremia and in pregnant women preterm delivery and even abortion (5). It is more common in women than men. Bacteremia is one of the emergencies. In certain infections, bacteremia is the best practical way to identify the disease-causing microorganism. *Klebsiella pneumoniae* usually form polysaccharide capsules (CPSs), also called K antigens, that cover the entire surface of the cell. One of the main characteristics of *Klebsiella pneumoniae* is the formation of mucoid colonies on a solid culture medium. When there is a lot of carbohydrates in the environment, the amount of polysaccharides also increases (6). *Klebsiella pneumoniae* develops an enzyme resistance called *Klebsiella pneumoniae* carbapenemase or *KPC*. Organisms containing carbapenemase can inactivate penicillin, cephalosporins, aztreonam, and carbapenem, and because carbapenems are specifically used to treat multidrug-resistant pathogens, resistance to this class of antibiotics is a global threat (7). Class A carbapenemases are phylogenetically divided into six groups, four of which are formed by the enzymes GES, *KPC*, IMI / NMC, and SME, while SHV-38 and SFC each form a separate group. Genes encoding class A carbapenemases can be located on either the chromosome or the plasmid. Among many members of the Enterobacteriaceae family, the genus Klebsiella containing *KPC* is responsible for high rates of antibiotic resistance in recent years due to high rates of antibiotic resistance to common antibiotics and plasmid transfer of these genes to other species and genera of Enterobacteriaceae. Separation and identification of strains with *KPC* are a real challenge for diagnostic laboratories today. Because carbapenems are the ultimate treatment for nosocomial infections caused by Gram-negative bacteria resistant to broad-spectrum cephalosporins, and so far no suitable alternative to their syllables has been found. The relationship between different studies in different parts of the world has been done in the study of Klebsiella strains producing *KPC* carbapenemase. *OXA*cillinases (*OXA*s) are also enzymes that encode resistance to carbapenems, cephalosporins such as ceftazidime, and monobactams (aztreonam). Class D *OXA*cillinases

have not been extensively studied in Class A and C, but this is changing due to the recent increase in the number of extensive clinical reports of pathogens exhibiting *OXA*-related resistance. *OXA*cillin beta-lactamases belong to class D in the Ambler classification, which is based on amino acid sequences, and have serine in their active position. These beta-lactamases are resistant to aminopenicillin and uridopenicillin (8).

The formation of mucoid colonies in *Klebsiella pneumoniae* is due to the presence of a thick layer of polysaccharide capsules that can absorb large amounts of water. The polysaccharide capsule is composed of 4 to 6 sugars, which in many cases are uronic acid. The capsule constituents cover the bacterial surface by a thick fibril-like structure with many layers (9). The capsule, on the one hand, prevents the bacterium from being phagocytosed by polymorphonuclear granulocytes, and on the other hand, from killing the bacterium by the lethal factors of the serum. Its molecular mechanism may involve inhibiting the activity or uptake of complement system components, particularly C3b. Recent research has identified about 82 capsule serotypes that are antigenically similar but different in polysaccharide skeletons. Despite antigenic diversity, monosaccharide units are limited in number, including L-focus, L-Ramanose, D-mannose, D-glucose, D-galactose, D-glucuronic acid, or D-galacturonic acid, in several companions. With O-steel and pyruvate (10). Serotypes K1 and K2 in *Klebsiella pneumoniae* cause liver abscess and increase pathogenicity. It has recently been shown that K1 is a major cause of primary liver abscess and causes metastasis, and K2 causes a secondary abscess. Serotypes K2, K4, K5 cause community-acquired pneumonia. Capsule serotypes K2, K7 and K33 are abundant in *Klebsiella pneumoniae* (11). The lipopolysaccharide (LPS) molecule is made up of lipid A, a polysaccharide nucleus, and a side chain called the O antigen. So far, 9 types of O antigen have been identified in *Klebsiella pneumoniae* , O: 1, O: 2, O: 3, O: 4, O: 5, O: 7, O: 8, O: 9, O: 12, which Type O: 1 is more common than other types. Natural human serum can kill bacteria due to the complement system. The alternative pathway is activated by bacteria in the absence of specific antibodies and plays an important role in killing bacteria compared to the classical pathway. Lipid A also activates the classical pathway in the absence of antibodies. Both complement pathways cause cell lysis by the membrane attack complex and by perforating the cell wall peptidoglycan and entering the bacterial inner membrane. Serum-resistant bacteria contain one or a combination of polysaccharide capsules, polysaccharide O side chains, and surface proteins (12). The wall components of gram-negative bacteria from inside the cell to the outside include the three main parts of the lipoprotein layer, the outer membrane, and lipopolysaccharide (LPS).

The lipoprotein layer (Brown lipoprotein), which binds the outer membrane layer to peptides and glycans via covalent bonding. Its protein component consists of 57 amino acids that bind to the DAP molecule by the amino acid lysine in the peptide tetrapeptide chain and its glycan component. Which consists of cysteine-binding thioglycerol, binds non-covalently to the outer membrane. This lipoprotein is found in all gram-negative bacteria except Pseudomonas aeruginosa. The outer membrane consists of two phospholipid layers. The aim of this study was to investigate the prevalence of *CTX-M*, *OXA* and *KPC* genes in *Klebsiella pneumoniae* isolates obtained from patients.

Materials and Methods

In this study, 63 samples of *Klebsiella pneumoniae* strain were collected from patients. Due to the importance of sterile culture media, solutions and glassware were sterilized by autoclave at 121 ° C and 15 psi for 15 minutes. Culture media used include Nutrient Agar, Simon Citrate, MR-VP, TSI, SIM, McConkey, EMB. Isolates were stored in a liquid medium with glycerol at minus 20 ° C. DNA extraction using Sinaclone DNA extraction kit (catalog number TGK1003).

The PCR was performed to identify genes using the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation (94°C for 60 s), annealing (54°C for 50 s), extension (72°C for 45 s) and final extension (72°C for 10 min) for *CTX-M* and *KPC* genes and initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 40 s), annealing (58°C for 40 s), extension (72°C for 1:30 min) and final extension (72°C for 5 min). Finally, the results were analyzed using 1% agarose gel for

electrophoresis of PCR products. All primers used in this study are listed in Table 1.

Table 1. Sequences of primers used for evaluation of gene expression.

	Sequence (5'->3')	
	Forward primer	Reverse primer
KPC	CGTTCTTGTCTC TCATGGCC	CCTCGCTGTGCTTGT CATCC
CTX-M	GCGTGATACCA CTTCACCTC	TGAAGTAAGTGACC AGAATC
OXA	TTGGTGGCATCG ATTATCGG	GAGCACTTCTTTTGT GATGGC

Statistical analysis

Graphs obtained from the data of the above experiments were performed using EXCEL 2010 software and statistical analysis, if necessary, was performed using SPSS 17 software. The mean of different groups was compared by Chi-square test at the significance level of 0.05. All experiments were performed in three replications.

Results

A total of 63 patients including 36 women (57.1%) and 27 men (42.9%) with an age range of 16 to 71 years and a mean age of 19 4 4.1 were included in the study.

Frequency of study samples

Out of 63 samples, 29 samples (46%) from urine, 15 samples (23.8%) from sputum, 9 samples (41.3%) from fecal samples, 5 samples (7.9%) from wound culture and 4 samples (6.3%) were obtained from intravascular catheter (CVP) of blood culture and 1 (1.6%) sample were obtained from cerebrospinal fluid (CSF). The frequency of samples collected in the present study based on gender is shown in Table 2 and Figure 1. The results showed that out of 29 urine samples collected, 18 samples were taken from women (62.1%) and 11 samples from men (37.9%).

Table 2. Frequency of study samples by gender.

Sample type	Female		Male	
	Number	percentages	Number	percentages
Urine	18	1.62	11	9.37
sputum	9	60	6	40
Feces	4	4.44	5	6.55
Cultivation of wounds	2	40	3	60
Blood culture	3	75	1	25
CSF	0	0	1	100

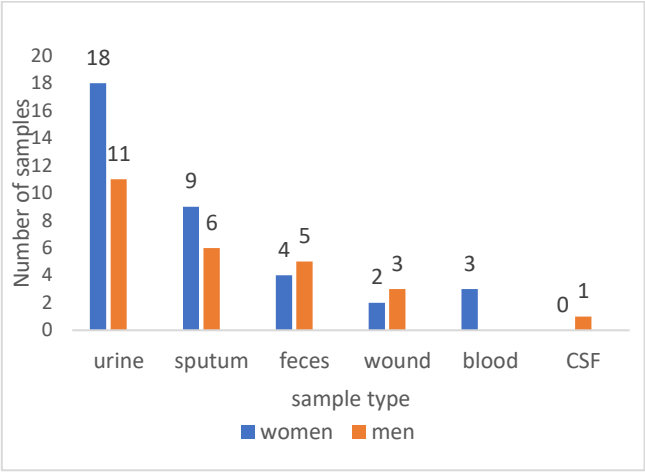


Figure 1. Results of biochemical tests of *Klebsiella pneumoniae* .

The results of biochemical tests of the studied strains and growth of *Klebsiella pneumoniae* colonies are shown in Table 3.

Table 3. Results of biochemical tests of *Klebsiella pneumoniae* strains.

Test	Result	Reaction
Gram staining and microscopic observation	Gram-negative bacilli	Red bacilli
Catalase test	Positive	Bubble production
Oxidase test	Negative	No change in reagent color
Simon Citrate test	Positive	Bromothymol blue reagent color changes from green to blue
TSI test	Negative	A / A and non-production of H_2S and production of CO_2 gas

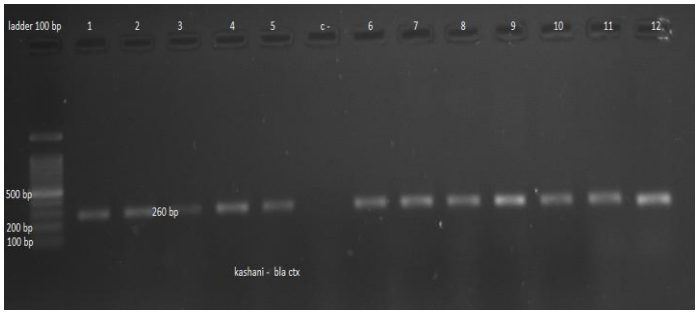
SIM test	Negative	Lack of movement and production of indole and H_2S
MR test	Negative	No red color
VP test	Positive	Red and pink color
Urease	Positive	Culture medium changes to pink or red
Lysine decarboxylase	Positive	Culture medium changes to purple

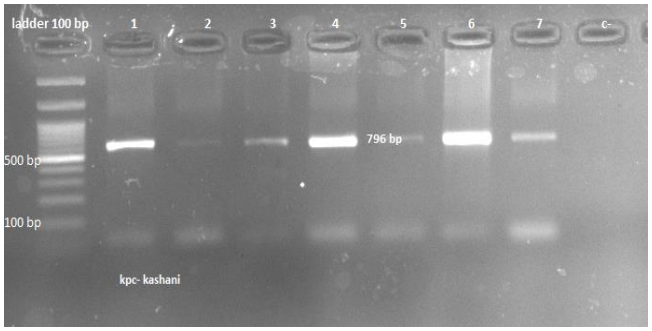
PCR reaction to evaluate the presence of *OXA*, *KPC* and *CTX-M* genes

The results of PCR test for the studied genes showed that 49 (77.8%), 49 (77.8%) and 46 (73%) strains carried *OXA*, *KPC* and *CTX-M* genes, respectively. Also 40 strains (63.5%) were positive for the presence of all genes under study. No genes were observed in 5 isolates (7.9%) (Table 4). Also, 5 (7.9%), 3 (4.8%) and 2 (3.2%) isolates, respectively, contained *OXA* / *KPC*, *OXA* / ctx and *KPC* / ctx genes simultaneously (Figure 2).

Table 4. Frequency of genes.

Strains	Genes						
	OXA	*KPC*	*CTX-M*	*OXA/KPC*	*OXA*/ctx	*KPC/ctx*	*Ctx/OXA/KPC*
Number	49	49	46	5	3	2	40
Percent	8.77	8.77	73	9.7	8.4	2.6	5.63





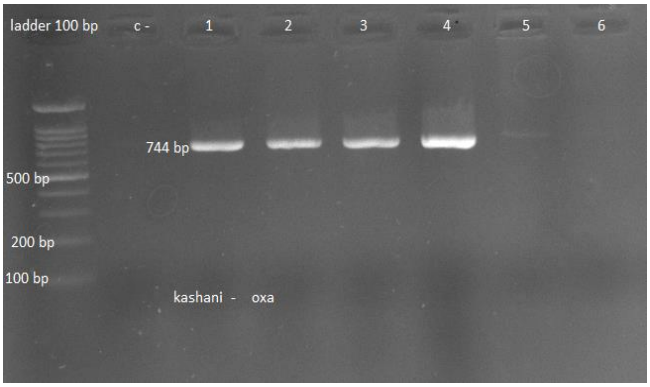


Figure 2. Electrophoresis of *CTX-M*, *KPC* and *OXA* gene on 1% agarose gel. C-negative control, columns 1 to 12 are the result of genes.

Discussion

Klebsiella pneumoniae or Friedlander bacillus is a gram-negative bacillus, has a capsule, urease positive and is a member of the Enterobacteriaceae family, which causes urinary tract infections, sepsis, pneumonia, abdominal, pelvic, etc. The presence of resistance genes in this bacterium has caused some therapeutic problems in recent years (13).

One of the most important antibiotics used today to treat infections caused by *Klebsiella pneumoniae* is the beta-lactam group of antibiotics, but unfortunately for reasons such as; Addition of antibiotics to the diet of cattle, improper, excessive and arbitrary use of antibiotics and lack of strict supervision in drug administration have led to the development of antibiotic-resistant strains (14). The main problem in the treatment of infections caused by these organisms is the emergence of strains with multiple resistance, which often leads to longer hospital stays, increased mortality and mobility, increased treatment costs compared to antibiotic-sensitive microbes, and ultimately treatment failure (7). Beta-lactamases are enzymes that inactivate these antibiotics by hydrolyzing the central nucleus of the beta-lactam ring. Ambler divided these enzymes into four groups (A-D) based on their initial structure: type B is metallobetalactamase, type C is cephalosporinase, and type A is broad-spectrum beta-lactamase (15). 1-*CTX-M* belongs to the ESBLs family and its gene is located on the plasmid and acts on cefotaxime (16). This enzyme can hydrolyze cephalosporins and is inhibited by clavulanic acid, sulbactam and tazobactam (17). The *CTX-M* family of broad-spectrum beta-lactamases was

first reported from Germany in 1989 and has since spread around the world. These enzymes are mainly present in the Enterobacteriaceae family (18). *CTX-M* beta-lactamases are not associated with TEM or HSV beta-lactamases and are only 14% similar to these two beta-lactamases. Unlike TEM and SHV beta-lactamases, *CTX-M* beta-lactamases have a greater hydrolyzing effect on cefotaxime and ceftriaxone antibiotics than on ceftazidime (19). Carbapenems are antibiotics of the beta-lactam family with bactericidal properties, and the cell wall of bacteria is one of the targets of these antibiotics, which leads to disruption of peptidoglycan synthesis (6, 10). In the present study, 63 strains of *Klebsiella pneumoniae* were collected from different clinical specimens such as blood, urine, sputum, feces, wounds, intravascular catheter and CSF. Most of the urine samples were isolated from women with urinary tract infections, which is consistent with the 2011 study by Salvatore et al. (20). The researchers found that urinary tract infections were more common in women than men, meaning that more than half of women developed UTIs at least once in their lifetime. Recurrence of the disease is common. Risk factors associated with a high frequency of UTI infections in women include female body anatomy, sexual intercourse, and family history. Hashemizadeh et al. (21) found that out of 202 collected Klebsiella strains (180 strains of *Klebsiella pneumoniae* and 22 strains of Klebsiella oxytocin), 22 isolates (11.9%) carried the *KPC* gene. Also, in contrast to the previous study, Aghasid Hosseini et al. (22) in Kashan in 2016 found that out of 181 collected Klebsiella, 21 (11.6%) carried the bla*KPC* gene, most of which were urinary and respiratory samples from patients admitted to the ICU. Which contradicts the present study. The results of the present study showed that out of 63 strains under study, 49 (77.8%) strains carried the *KPC* gene. Reasons for the discrepancy include geographical distance, a pattern of drug use in the hospitals under study, and microbial genetics. On the other hand, in agreement with the present study, Castanira et al. (23) and Chen et al. (24) found that the distribution of bla*KPC* gene in *Klebsiella pneumoniae* strains was 76% and 73.5%, respectively. This understanding may be due to the origin of the samples from the two studies and the use of similar primers. In the study, Hashemizadeh et al. Found that the origin of the samples could alter the genetic pattern of the microbe. These researchers showed that most of the positive cases included hospital samples (66.8%) and outpatients (33.2%). The *OXA*-48 gene is found in plasmids containing NDM. The size of this plasmid is about 62.5 kb. This enzyme has been isolated from hospitalized patients in France, Germany, Spain, and the United Kingdom, and, as mentioned earlier, uses molecular methods to identify the gene. In the present study, 77.8% (49 strains) carried the *OXA*-48 gene, which is consistent with the study of Machuca et al. (25). In their study in Isfahan in 2020, Solgi et al. (26) found that 66.2% of *Klebsiella pneumoniae* strains carried the *OXA*-48 gene. Contrary to the current study, Hosseinzadeh et al. (27) found in 2018 that out of 211 strains of *Klebsiella pneumoniae* , only 2 isolates (0.9%) carried the *OXA*-48 gene. This discrepancy may be due to differences in sample type and geographical distance. On the other hand, the results of the molecular analysis showed that 73% (46 isolates) carried *CTX-M* genes, which is contrary to the study of Lashgari et al. (28) per year. Lashgari et al. In 2014, in a study entitled Molecular detection of beta-lactamase gene bla*CTX-M* in *Klebsiella pneumoniae* strains isolated from clinical samples, found that out of 100 samples of *Klebsiella pneumoniae* , 46 isolates carried the gene. This discrepancy may be due to differences in the year of the study.

Conclusion

Due to the high prevalence of *Klebsiella pneumoniae* and the reported resistance genes of this bacterium, they may be mistakenly considered sensitive in routine laboratory phenotypic tests and may develop and develop more resistant pathogens by prescribing ineffective antibiotics. On the other hand, there is insufficient information about the frequency of this plasmid gene and its genetic pattern in Iran. Therefore, detection of *Klebsiella pneumoniae* strains containing beta-lactamase resistance enzymes is important for better treatment and prevention of the spread of these genes to other bacteria using accurate phenotypic and genotypic methods.

Author contributions

MA managed the project and wrote and revised the manuscript.**GhK** collected the date and did the experimental tests.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791.
2. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis. 2014;20(7):1170.
3. Fraenkel-Wandel Y, Raveh-Brawer D, Wiener-Well Y, Yinnon AM, Assous MV. Mortality due to bla KPC Klebsiella pneumoniae bacteraemia. J Antimicrob Chemother. 2016;71(4):1083-7.
4. Wiener-Well Y, Raveh-Brawer D, Fraenkel-Wandel Y, Yinnon AM, Assous MV. Mortality due to bla KPC Klebsiella pneumoniae bacteraemia—authors' response. J Antimicrob Chemother. 2016;71(6):1744-.
5. Giacobbe DR, Tumbarello M, Del Bono V, Viscoli C. Comment on: Mortality due to bla KPC Klebsiella pneumoniae bacteraemia. J Antimicrob Chemother. 2016;71(6):1743-4.
6. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1-9.
7. Daikos GL, Petrikkos P, Psichogiou M, Kosmidis C, Vryonis E, Skoutelis A, et al. Prospective observational study of the impact of VIM-1 metallo-β-lactamase on the outcome of patients with Klebsiella pneumoniae bloodstream infections. Antimicrob Agents Chemother. 2009;53(5):1868-73.
8. Lodise TP, Ye M, Keyloun KR, Zhao Q, Gillard P. Identification of patients at greatest risk for carbapenem resistance in patients with serious hospital-onset infections due to Enterobacteriaceae species. Open Forum Infect Dis. 2016;3(suppl_1):1794.
9. Walther-Rasmussen J, Høiby N. Class A carbapenemases. J Antimicrob Chemother. 2007;60(3):470-82.
10. Vardakas KZ, Rafailidis PI, Konstantelias AA, Falagas ME. Predictors of mortality in patients with infections due to multi-drug resistant Gram negative bacteria: the study, the patient, the bug or the drug? J Infect. 2013;66(5):401-14.
11. Castón JJ, Lacort-Peralta I, Martín-Dávila P, Loeches B, Tabares S, Temkin L, et al. Clinical efficacy of ceftazidime/avibactam versus other active agents for the treatment of bacteremia due to carbapenemase-producing Enterobacteriaceae in hematologic patients. Int J Infect Dis. 2017;59:118-23.
12. Ben-David D, Kordevani R, Keller N, Tal I, Marzel A, Gal-Mor O, et al. Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. Clin Microbiol Infect. 2012;18(1):54-60.
13. Karaiskos I, Giamarellou H. Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. Expert Opin Pharmacother. 2014;15(10):1351-70.
14. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother. 2015;59(10):5873-84.
15. Liu S-W, Chang H-J, Chia J-H, Kuo A-J, Wu T-L, Lee M-H. Outcomes and characteristics of ertapenem-nonsusceptible Klebsiella pneumoniae bacteremia at a university hospital in Northern Taiwan: a matched case-control study. J Microbiol Immunol Infect. 2012;45(2):113-9.
16. Bartoletti M, Giannella M, Caraceni P, Domenicali M, Ambretti S, Tedeschi S, et al. Epidemiology and outcomes of bloodstream infection in patients with cirrhosis. J Hepatol. 2014;61(1):51-8.
17. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, et al. Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase–producing K. pneumoniae: importance of combination therapy. Clin Infect Dis. 2012;55(7):943-50.
18. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177-88.
19. Gallagher JC, Kuriakose S, Haynes K, Axelrod P. Case-case-control study of patients with carbapenem-resistant and third-generation-cephalosporin-resistant Klebsiella pneumoniae bloodstream infections. Antimicrob Agents Chemother. 2014;58(10):5732-5.
20. Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P, et al. Urinary tract infections in women. Eur J Obstet Gynecol Reprod Biol. 2011;156(2):131-6.
21. Mirnejhad R, Hashemizadeh FS, Zamanzad B, Jahandideh S, Ansari N, Gholipour A, et al. Identification of KPC-producing Klebsiella

pneumoniae in clinical samples in Iran. Yafte journal of medical scienes (YJMS). 2013;15(1).
22. Agha-Seyed Hosseini M, Firoozeh F, Piroozmand A, Gilasi HR. Carbapenemase-producing Klebsiella pneumoniae strains among clinical specimens in Kashan (2014-2015). Feyz. 2016;20(3):267-73.
23. Castanheira M, Sader HS, Deshpande LM, Fritsche TR, Jones RN. Antimicrobial activities of tigecycline and other broad-spectrum antimicrobials tested against serine carbapenemase-and metallo-β-lactamase-producing Enterobacteriaceae: report from the SENTRY Antimicrobial Surveillance Program. Antimicrob Agents Chemother. 2008;52(2):570-3.
24. Chen S, Hu F, Xu X, Liu Y, Wu W, Zhu D, et al. High prevalence of KPC-2-type carbapenemase coupled with CTX-M-type extended-spectrum β-lactamases in carbapenem-resistant Klebsiella pneumoniae in a teaching hospital in China. Antimicrob Agents Chemother. 2011;55(5):2493-4.
25. Machuca J, López-Cerero L, Fernández-Cuenca F, Mora-Navas L, Mediavilla-Gradolph C, López-Rodríguez I, et al. OXA-48-like-producing Klebsiella pneumoniae in Southern Spain in 2014–2015. Antimicrob Agents Chemother. 2019;63(1):e01396-18.
26. Solgi H, Nematzadeh S, Giske CG, Badmasti F, Westerlund F, Lin Y-L, et al. Molecular epidemiology of OXA-48 and NDM-1 producing enterobacterales species at a University Hospital in Tehran, Iran, between 2015 and 2016. Front Microbiol 2020;11:936.
27. Hosseinzadeh Z, Ebrahim-Saraie HS, Sarvari J, Mardaneh J, Dehghani B, Rokni-Hosseini SMH, et al. Emerge of bla NDM-1 and bla OXA-48-like harboring carbapenem-resistant Klebsiella pneumoniae isolates from hospitalized patients in southwestern Iran. J Chin Med Assoc. 2018;81(6):536-40.
28. Lashgari N, Vand Yousefi J, Siadat SD, Shahcheraghi F, Khosravi M, Vakili H, et al. Identification of bla-CTX-M β-lactamase in Klebsiella pnumoniae clinical isolates by polymerase chain reaction. Med Sci J Islamic Azad Univ Tehran Med Branc. 2014;24(3):148-52.