DOI: https://doi.org/10.53350/pjmhs211572292

ORIGINAL ARTICLE

Detection of KPC-2 gene in K. pneumoniae, E. coli and P. mirabilis isolated from Urine sample of Urinary Tract Infection patients

NARGIS[1], TAYYAB UR REHMAN[2], LIAQAT ALI[3], HANIF KHAN[4], MADINA[5]
[1]*MPhil Scholar,* Khyber Medical University *(*KMU*), Peshawar*
[2, 4]*Resident Urologist, Institute of Kidney Disease (IKD), Hayatabad Peshawar*
[3]*Associate Professor, Institute of Kidney Disease (IKD), Hayatabad Pes hawar*
[5]*Medical Student at KMC, Peshawar*
Corresponding Author: Dr. Liaqat Ali, Email: liqat_99@yahoo.com, Cell: +923409666665

ABSTRACT

Background: Carbapenem resistance in Enterobacteriaceae is an uprising problem worldwide. KPC is one of the important mechanisms of resistance in Enterobacteriaceae such as K. pneumoniae.

Aims and Objectives: The current research focuses on the frequency of the KPC -2 gene in Enterobacteriaceae isolated from urine samples, as well as antibiotic resistance patterns.

Methodology: Antibiotic sensitivity patterns were examined on 53 carbapenem-resistant isolates from the Enterobacteriaceae family. These isolates were subjected to the Modified Hodge Test (MHT) and PCR for KPC 2 gene identification.

Results: A total of 150 urine samples were processed for the isolation of the most prevalent Enterobacteriaceae. 125 Gram-negative bacterial isolates were obtained in which the consistency of K. pneumonia was 50(40%),E. colin was 55(44%), and P. mirabilis was 20(16%). The test for susceptibility of antibioticresulted that among50 Klebsiella pneumoniae 40% were resistant to Imipenem, while in E. coli 54.4% and P. mirabilis 30 % were resistant to Imipenem respectively. PCR results show the gene KPC-2 out of 15 (75%) 2 (13.2%) Modified Hodge Test Positive Klebsiella pneumoniae isolates. In total 83.3% (n=25) E. coli Modified Hodge Test positive and for the KPC-2 gene 4% were positive.

Conclusion: This research demonstrates that in Enterobacteriaceae there isexistence of carbapenem resistance. Surveillance research and complete antibiotic prescription standards should be established at Pakistan's various hospitals to stop the growth of antibiotic-resistant bacteria.

Key Words: Enterobacteriaceae, Urinary Tract Infections, Carbapenem, Modified Hodge test

INTRODUCTION

Enterobacteriaceae, the bacteria that cause urinary tract infections in the community and in hospitals, have evolved progressively multidrug resistance to antibiotics (both first and second lines) (Nordmann et al., 2011).Thus far, carbapenems were thought to be the last option for treating infections caused by ESBL-producing Enterobacteriaceae.Unfortunately, the emergence of Carbapenem-Resistant Enterobacteriaceae (CRE) is highly associated with carbapenem usage(Harris et al., 2015)(Chang et al., 2011)(Loon van et al., 2018).

Carbapenem antibiotics belong to the beta-lactam group of antibiotics and have a broad spectrum of activity against gram-positive and gram-negative bacteria(Meletis, 2016). Resistant to carbapenem antibiotics in gram-negative bacteria is due to either alteration of porin, upregulation of efflux pump, production of Carbapenemase enzymes(Drawz & Bonomo, 2010). KPC-type carbapenemases are the foremost cause of resistance in Klebsiella pneumoniae and in many other Enterobacteriaceae (Nordmann et al., 2011)(Tzouvelekis et al., 2012). Klebsiella pneumoniae carbapenemases (KPCs) are ß-lactamases produced by Gram-negative bacteria. They efficiently hydrolyze penicillins, all cephalosporins, monobactams, carbapenems, and even ß-lactamase inhibitors (Munoz-Price et al., 2013)

In 2001 first “Klebsiella pneumoniae carbapenemase” (KPC) was discovered, which is present in carbapenem-resistant Klebsiella pneumoniae in the USA (Yigit et al., 2001). To date, more than39 KPC variants have been described (Naas et al., 2017). Tn4401 transposon contains the blaKPC gene.Which is found on plasmids of various sizes and replicon types, or is incorporated into the bacterial chromosome (Mathers et al., 2017).Interspecies transmission during epidemics has been observed in hospitalized patients. The KPC gene may transfer to bacteria other than K. pneumoniae, such as Enterobacter spp., Citrobacter spp., or E. coli. (Mathers et al., 2017)(Tzouvelekis et al., 2012). Carbapenem resistance between Enterobacteriaceae is a huge public health problem due to its fast worldwide spread (Eichenberger & Thaden, 2019).

To minimise the spread of KPC harboring bacteria, efficientmeans to control infection, and precise use of antibiotic should be accompanied with the application of fast and efficient KPC diagnostic test (Hindiyeh et al., 2008; Raghunathan et al., 2011).

In the present study we Isolated and Identified Antibiotic Sensitivity Pattern of Bacteria from Urine Samples of UTI patients, as suggested by the Clinical and Laboratory Standards Institute (CLSI),, to establish the existence of carbapenemase activity , Phenotypic techniques, such as the modified Hodge test (MHT), were used to identify KPCs. The KPC gene was discovered and identified using the polymerase chain reaction (PCR).

MATERIAL AND METHODS

This research was carried out in the IBMS – KMU Peshawar microbiology laboratory in collaboration of institute of kidney diseases Hayatabad Peshawar.

Sampling and isolation: The samples of urine were obtained from the labs of Peshawar's tertiary care

institutions. Quantitative isolation was used to inoculate the urine sample on McConkey and CLED medium.The plates were inverted and put in an incubator set to 37 °C. The test which were used in the identification of isolates are triple sugar iron test, indole test, oxidase test, colony morphology, urease test and Gram staining were used to identify isolates.

Antibiotic susceptibility: Antibiotic susceptibility was performed using the disc diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute Antibiotic susceptibility was performed against aztreonam (ATM;30 $x10^{-6}$g), Ampicillin (AMP;10 $x10^{-6}$g), amikacin (AK;10 $x10^{-6}$g), cefoxitin (FOX; 30 $x10^{-6}$g), and imipenem (IMP;10 $x10^{-6}$g), cefepime (FEP;10 $x10^{-6}$g)(CLSI, 2017).

Modified Hodge Test: According to the Clinical Laboratory Standards Institute (CLSI) 2017 recommendations, carbapenemase detection of isolates was done using the Modified Hodge test(MHT) with Mueller – Hinton agar (MHA). (CLSI, 2017).

DNA extraction and visualization: Extraction OF Plasmid DNA was done with alkaline lysis technique (Feliciello & Chinali, 1993).

A microvolume spectrometer (Colibri, Titertek Berthold) was used to quantify the extracted DNA, and its quality was evaluated on a 1.5% agarose gel stained with ethidium bromide.

PCR analysis of KPC-2 gene: KPC-2 was detected through PCR by using gene-specific primers KPC F (TCCGTTACGGCAAAAATGCG) KPC R (CGGCATAGTCATTTGCCGTG), constructed with the help of the KPC-2 generating sequence ofKlebsiella pneumoniae (GenBank accession no. AY034847).

PCR Reaction was performed in 20 µl reaction volume. Green Taq Master Mix, nuclease-free water, 0.5 $x10^{-6}$l forward primer, 0.5 $x10^{-6}$l reverse primer, and 1 $x10^{-6}$l of DNA were used to make this reaction mixture. After that, the mixture was maintained in a PCR machine (BioRad, Thermal Cycler Model # I Cycler) for a while. The conditions of PCR were as initial denaturation was done for 5 minutes at 95 ° C , afterwards at 94 ° C 35 cycles of denaturation. Then annealing was done for 1 minute at 56 ° C and extension for 35 seconds at 72 ° C. A 5-minute final extension operation was performed at 72 C.PCR product was than observe on 1.5% gel under UV transilluminator.

RESULTS

One hundred twenty-five Enterobacteriaceae were isolated from the urine sample. These 125 isolates consist of 50 (40%) K. pneumoniae 55 (44%) E. coli and 20 (16%) P. mirabilis. The antibiotic susceptibility pattern is presented in Table 1. In 50 K. pneumoniae isolates 40%, 56%, 52%, 48%, 58%, 48% were resistant to imipenem, ampicillin, cefepime, cefoxitin, aztreonam, and amikacin respectively. Among 55 E. coli isolate 54.4% were resistant to imipenem, 67.27 % to Ampicillin, 61.81% to cefepime , 65% to cefoxitin, 76.36% to aztreonam and 70.9% to amikacin. In P. mirabilis isolates 30% resistant to imipenem, 5% to Ampicillin, 10% to cefepime, 15% to cefoxitin, 20% to aztreonam, 25% to amikacin.Out of 125 isolates, 53 were resistant to imipenem which includes 40 % (20) K. pneumonia, (30) 54.4% E. coli, and (3)30% P. mirabilis.

In 20 carbapenem-resistant K. pneumonia 17 of it shows positive inmodified hodge test and in other study of 30 carbapenem-resistant E. coli, 25 were positive in modified hodge test. While 2 were positive in modified hodge test among 3 carbapenem-resistant P. mirabilis 2 were MHT positive. Overall 42 were positive in modified hodge test among 53 carbapenem-resistant isolates Table 2.

PCR result shows that 2 K. pneumoniae and 1 E. coli harbor the KPC-2 gene Figure 1 -Figure 2. while no KPC-2 gene indicated in P. mirabilis Table 3.

Table 1: Antibiotic Resistant Pattern of all isolates examined in the study

Name of Organism	Imipenem	Ampicillin	Cefepime	Cefoxitin	Aztreonam	Amikacin
K. pneumoniae n=50	40%	56%	52%	48%	58%	48%
E. coli n=55	54.4%	67.27%	61.81%	65%	76.36%	70.9%
P. mirabilis n= 20	30%	5%	10%	15%	20%	25%

Table 2: Modified Hodge test results of all isolates included in the study

S.no	Carbapenem Resistant bacteria	Positive MHT	Percentage
1	K. pneumoniae N=20	15	75%
2	E. coli n=30	25	83.3%
3	P. mirabillis n= 3	2	66.6%
Total	53	42	79.24%

Table 3 PCR results of MHT positive Isolates

S.no	Isolates Name	Positive MHT	Isolated harboring KPC-2 gene	Percentage
1	K. pneumoniae	15	2	13.2%
2	E. coli	25	1	4%
3	P. mirabilis	2	0	0%
Total		42	4	9.5 %

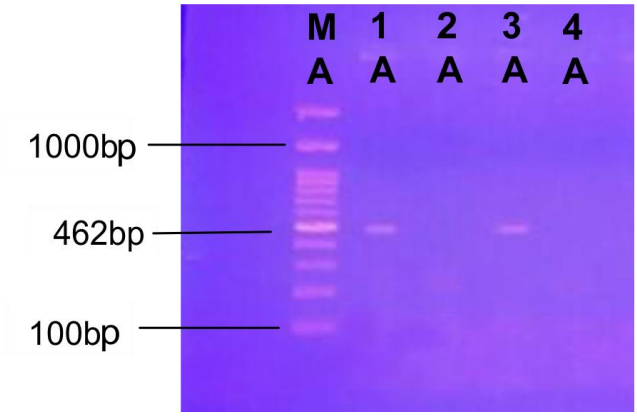

Figure 1: Gel electrophoresis of PCR amplified of KPC-2 gene at 462bp.Lane(M) molecular maker (100bp DNA ladder), Lane (1) PCR product of K. pneumonia no.23, Lane (2) negative PCR product, Lane (3) PCR product of K. pneumoniae no.62, lane (4) negative control

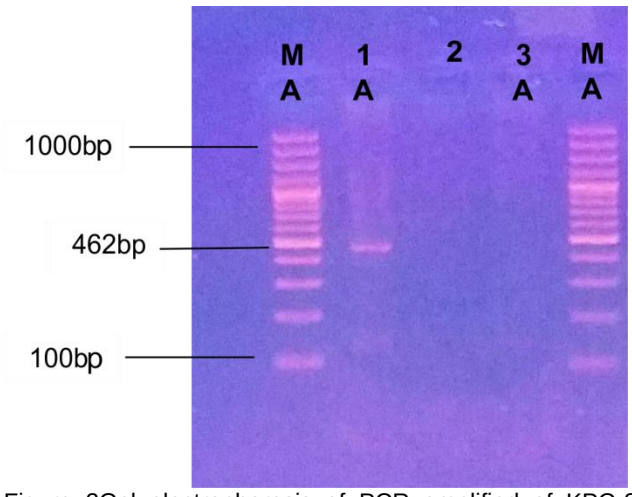

Figure 2Gel electrophoresis of PCR amplified of KPC-2 gene at 462bp. Lane(M) both on left and right-side molecular maker (100bp DNA ladder), Lane (1) PCR product of E. coli no.23, Lane (2) negative PCR product, lane (3) negative control

DISCUSSION

The proliferation of carbapenamase-producing bacterial strains isolated from urine samples from UTI patients in various health centers (governmental and non - governmental hospitals in Peshawar) that exhibited resistance to carbapenem antibiotic susceptibility to imipenem was shown in this research. The rising frequency of the clinical MDR-KPC phenotype has been linked to increased mortality rate, presenting a significant public health concern (Mezzatesta et al., 2011).

In the present study 125 bacterial isolates were obtained from urine samples of UTI patients.

The obtained bacteria consist of 55(44%) E. coli, 50(40%) K. pnuemoniae, and 20(16 %) P. mirabilis respectively. A study done by Fridullal et al reported that in total 90 isolates from urine sample 14 % were K. pneumonia and 53%were E. coli(Muhammad et al., 2018). Taj bakhsh E et al performed yet another research in Iran resulted that the proportion was 51.70 % of E. coli, 16.32 % of K. pneumonia and 10.88 % of P. mirabilis (Tajbakhsh et al., 2015). There is a distinction in isolated pathogens between our and the other studies due to species variations and geographical distribution.

In the present study 40 %K. pneumonia showed resistance to imipenem while E. coli and P. mirabilis showed 54.4 % and 30% resistance respectively. Francis RO et al saidin his study that in K. pneumoniae the rate of carbapenem-resistant was 29% and in E. coli 2.8% (Francis et al., 2012). According to a 2013 research by Priyadarshini, the percentage of resistance against imipenem of K. pneumoniae was 77 % (Shanmugam et al., 2013). Some other research by Nazir et al. found that 21% of K. pneumonia and 9% of E. coli were carbapenem resistant (Nazir et al., 2011). The difference in resistant rate in our study and other study is due to the difference in the type of samples and the number of bacterial isolates.

In the current study, we found that 15 (75%) of 20 carbapenem-resistant K. pneumoniae were Modified Hodge Test positive, whereas 25 (83.3%) of 30 carbapenem-resistant E. coli were Modified Hodge Test positive, and only 2 (66.6%) of 3 carbapenem-resistant P. mirabilis were Modified Hodge Test positive. Only 79.24 % of the 53 carbapenem Enterobacteriaceae tested positive for Modified Hodge Test. Sanjeev Kumar et al. reported in his studies that among 50 carbapenem-resistant Enterobacteriaceae 17 (34%) were Modified Hodge Testpositive(Kumar & Mehra, 2015). Research heldin Brazil shows that carbapenem-resistant Enterobacteriaceae 784 (83.27%) were Modified Hodge Test positive from total 942 (Arend et al., 2015). A study was done by Bina et al in that 80.5% (33 of 41) isolates were Modified Hodge Test positive (Bina et al., 2015). The variation in the ratio of MHT positive isolates may be due to the difference in the sample source from which carbapenem-resistant bacteria were isolated. We isolated Enterobacteriaceae from the patients UTI using urine samples.

The KPC-2 gene was found in two2 (13.2%) of the 15 MHT positive K. pneumoniae in this research. In the case of 25 (83.3%) MHT positive E. coli, 1 (4%) carried the KPC-2 gene. Priyadarshini Shanmugam et al found that 28 (67.4%) of the 38 MHT positive isolates contained the KPC-2 gene (Shanmugam et al., 2013). Sattar et al. (2014) showed that 30% of K. pneumoniae had the KPC-2 gene, whereas the frequency of same gene in E. coli was 14.6 percent (Sattar et al., 2016).

The limited sample size explains the differential in prevalence. False-positive MHT findings are also more frequent in isolates that produce AmpC and CTX-M b-lactamases. MHT is only able to identify the presence of carbapenemase enzyme in the positive sample, not the particular Carbapenemase generated by the isolates.

In Pakistan, resistance in Gram-negative organisms was increasingly recognized. A study was done by Aga Khan University, Karachi from 2001-2006 showed an increase in ESBL producing K. pneumoniae to >30% and 0.4% Carbapenem resistance. Pakistan develops a national action plan for the containment of antibiotic resistance, major aspects of this plan is to improve

awareness on antimicrobial resistance, reduce the incidence of infection, modify the use of antimicrobial agents (Plan, 2017).

CONCLUSION

In this research, the KPC-2 gene was discovered in K. pneumoniae and E. coli isolates from urine. As a consequence, early detection of carbapenem-resistant bacteria is essential for effective treatment and infection control measures to prevent the germs from spreading between patients and inside institutions.

REFERENCES

1. Arend, L. N., Pilonetto, M., de Alencar Siebra, C., & Tuon, F. F. (2015). Phenotypic and molecular characterization of 942 carbapenem-resistant Enterobacteriaceae (CRE) in southern Brazil. Journal of Infection and Chemotherapy, 21(4), 316–318. https://doi.org/10.1016/j.jiac.2014.11.011
2. Bina, M., Pournajaf, A., Mirkalantari, S., Talebi, M., & Irajian, G. (2015). Detection of the Klebsiella pneumoniae carbapenemase (KPC) in K.pneumoniae isolated from the clinical samples by the phenotypic and genotypic methods. Iranian Journal of Pathology, 10(3), 199–205. https://doi.org/10.7508/ijp.2015.03.004
3. Chang, H. J., Hsu, P. C., Yang, C. C., Kuo, A. J., Chia, J. H., Wu, T. L., & Lee, M. H. (2011). Risk factors and outcomes of carbapenem-nonsusceptible Escherichia coli bacteremia: A matched case-control study. Journal of Microbiology, Immunology and Infection, 44(2), 125–130. https://doi.org/10.1016/j.jmii.2010.06.001
4. CLSI. (2017). Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. In Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.
5. Drawz, S. M., & Bonomo, R. A. (2010). Three decades of β-lactamase inhibitors. Clinical Microbiology Reviews, 23(1), 160–201. https://doi.org/10.1128/CMR.00037-09
6. Eichenberger, E. M., & Thaden, J. T. (2019). Epidemiology and mechanisms of resistance of extensively drug resistant gram-negative bacteria. Antibiotics, 8(2). https://doi.org/10.3390/antibiotics8020037
7. Feliciello, I., & Chinali, G. (1993). A modified alkaline lysis method for the preparation of highly purified plasmid DNA from Escherichia coli. In Analytical Biochemistry (Vol. 212, Issue 2, pp. 394–401). https://doi.org/10.1006/abio.1993.1346
8. Francis, R. O., Wu, F., Della-Latta, P., Shi, J., & Whittier, S. (2012). Rapid detection of Klebsiella pneumoniae carbapenemase genes in enterobacteriaceae directly from blood culture bottles by real-time PCR. American Journal of Clinical Pathology, 137(4), 627–632. https://doi.org/10.1309/AJCP9SNHJG2QGLWU
9. Harris, P. N. A., Tambyah, P. A., & Paterson, D. L. (2015). β-lactam and β-lactamase inhibitor combinations in the treatment of extended-spectrum β-lactamase producing Enterobacteriaceae: Time for a reappraisal in the era of few antibiotic options? The Lancet Infectious Diseases, 15(4), 475–485. https://doi.org/10.1016/S1473-3099(14)70950-8
10. Hindiyeh, M., Smollen, G., Grossman, Z., Ram, D., Davidson, Y., Mileguir, F., Vax, M., Ben David, D., Tal, I., Rahav, G., Shamiss, A., Mendelson, E., & Keller, N. (2008). Rapid detection of blaKPC carbapenemase genes by real-time PCR. Journal of Clinical Microbiology, 46(9), 2879–2883. https://doi.org/10.1128/JCM.00661-08
11. Kumar, S., & Mehra, S. K. (2015). Performance of Modified Hodge Test and Combined Disc Test for Detection of Carbapenemases in Clinical Isolates of Enterobacteriaceae. Int.J.Curr.Microbiol.App.Sci, 4(5), 255–261. https://www.ijcmas.com/vol-4-5/Sanjeev Kumar and S.K.Mehra.pdf
12. Loon van, K., Voor in 't holt, A. F., & Vos, M. C. (2018). A Systematic Review and Meta-analyses of the Clinical. Antimicrobial Agents and Chemotherapy, 62(e01730-17), 1–18. https://doi.org/10.1128/AAC.01730-17
13. Mathers, A. J., Stoesser, N., Chai, W., Carroll, J., Barry, K., Cherunvanky, A., Sebra, R., Kasarskis, A., Peto, T. E., Walker, A. S., Sifri, C. D., Crook, D. W., & Sheppard, A. E. (2017). Chromosomal integration of the Klebsiella pneumoniae carbapenemase gene, blaKPC, in Klebsiella species is elusive but not rare. Antimicrobial Agents and Chemotherapy, 61(3). https://doi.org/10.1128/AAC.01823-16
14. Meletis, G. (2016). Carbapenem resistance: overview of the problem and future perspectives. Therapeutic Advances in Infectious Disease, 3(1), 15–21. https://doi.org/10.1177/2049936115621709
15. Mezzatesta, M. L., Gona, F., Caio, C., Petrolito, V., Sciortino, D., Sciacca, A., Santangelo, C., & Stefani, S. (2011). Outbreak of KPC-3-producing, and colistin-resistant, Klebsiella pneumoniae infections in two Sicilian hospitals. Clinical Microbiology and Infection, 17(9), 1444–1447. https://doi.org/10.1111/j.1469-0691.2011.03572.x
16. Muhammad, D., Ishaq, M. S., Zoreen, A., & Farooq, S. (2018). Abasyn Journal of Life Sciences Isolation and Identification of Pathogenic Bacteria from Urinary Tract Infections. 1(1), 19–25.
17. Munoz-Price, L. S., Poirel, L., Bonomo, R. A., Schwaber, M. J., Daikos, G. L., Cormican, M., Cornaglia, G., Garau, J., Gniadkowski, M., Hayden, M. K., Kumarasamy, K., Livermore, D. M., Maya, J. J., Nordmann, P., Patel, J. B., Paterson, D. L., Pitout, J., Villegas, M. V., Wang, H., … Quinn, J. P. (2013). Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. The Lancet Infectious Diseases, 13(9), 785–796. https://doi.org/10.1016/S1473-3099(13)70190-7
18. Naas, T., Oueslati, S., Bonnin, R. A., Dabos, M. L., Zavala, A., Dortet, L., Retailleau, P., & Iorga, B. I. (2017). Beta-lactamase database (BLDB)–structure and function. Journal of Enzyme Inhibition and Medicinal Chemistry, 32(1), 917–919. https://doi.org/10.1080/14756366.2017.1344235
19. Nazir, H., Cao, S., Hasan, F., & Hughes, D. (2011). Can phylogenetic type predict resistance development? Journal of Antimicrobial Chemotherapy, 66(4), 778–787. https://doi.org/10.1093/jac/dkq505
20. Nordmann, P., Naas, T., & Poirel, L. (2011). Global spread of carbapenemase producing Enterobacteriaceae. Emerging Infectious Diseases, 17(10), 1791–1798. https://doi.org/10.3201/eid1710.110655
21. Plan, N. A. (2017). National AMR Action Plan for Pakistan Antimicrobial Resistance National Action Plan Pakistan Ministry of National Health Services Regulations & Coordination Government of Pakistan. May, 1–64. https://www.nih.org.pk/wp-content/uploads/2018/08/AMR-National-Action-Plan-Pakistan.pdf
22. Raghunathan, A., Samuel, L., & Tibbetts, R. J. (2011). Evaluation of a real-time PCR assay for the detection of the klebsiella pneumoniae carbapenemase genes in microbiological samples in comparison with the modified hodge test. American Journal of Clinical Pathology, 135(4), 566–571. https://doi.org/10.1309/AJCPPVNUI3O9JHJW
23. Sattar, H., Toleman, M., Nahid, F., & Zahra, R. (2016). Co-existence of blaNDM-1 and blaKPC-2 in clinical isolates of Klebsiella pneumoniae from Pakistan. Journal of Chemotherapy, 28(4), 346–349. https://doi.org/10.1179/1973947814Y.0000000223
24. Shanmugam, P., Meenakshisundaram, J., & Jayaraman, P. (2013). BlaKPC gene detection in clinical isolates of carbapenem resistant Enterobacteriaceae in a tertiary care hospital. Journal of Clinical and Diagnostic Research, 7(12), 2736–2738. https://doi.org/10.7860/JCDR/2013/7759.3747
25. Tajbakhsh, E., Tajbakhsh, S., & Khamesipour, F. (2015). Isolation and molecular detection of gram negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. Iranian Red Crescent Medical Journal, 17(5). https://doi.org/10.5812/ircmj.17(5)2015.24779
26. Tzouvelekis, L. S., Markogiannakis, A., Psichogiou, M., Tassios, P. T., & Daikos, G. L. (2012). Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. Clinical Microbiology Reviews, 25(4), 682–707. https://doi.org/10.1128/CMR.05035-11
27. Yigit, H., Queenan, A. M., Anderson, G. J., Domenech-sanchez, A., Biddle, J. W., & Steward, C. D. (2001). Novel Carbapenem-Hydrolyzing ␤-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of. Society, 45(4), 1151–1161. https://doi.org/10.1128/AAC.45.4.1151