



DETECTION OF bla_{OXA-10} AND bla_{SHV} GENES FROM *KLEBSIELLA PNEUMONIAE* ISOLATED FROM IRAQI UTI PATIENTS BY REAL TIME-PCR AND CONVENTIONAL PCR

Firas Nabeeh Jaafar, Mohammed Twfeek Abid Alhusain and Afrah Abdulridha Ajeel Hassan*

Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq.

*Corresponding Author: afrahalmaliki79@uomustansiriya.edu.iq

Abstract

Enterobacteriaceae is generating extended-spectrum β -lactamase (ESBL) which leads to resistance to β -lactam antibiotics. *K. pneumoniae* is, member of the family of Enterobacteriaceae, considered as one of the most widely recognized causative agents of urinary tract infection (UTI). The detection of β -lactamase in *K. pneumoniae* is rarely reported in Iraq. The study's objective was, therefore, to detect the genes of bla_{SHV} and bla_{OXA-10} from *K. pneumoniae* by conventional PCR and real-time PCR (RT-PCR). Urine samples were collected from patients severing UTI infections and cultured on MacConkey and UTI agar. Molecular detection of *K. pneumoniae* isolates was determined by conventional PCR and RT-PCR. The analysis of RT-PCR was made by collecting the value of melting temperature. Among the 19 strains of *K. pneumoniae*, 15 were found to carry bla_{SHV} gene and 16 were found to carry bla_{OXA-10} gene by conventional PCR. In respect to RT-PCR, all the strains were detected to have bla_{SHV} and bla_{OXA-10} . Melting curve of bla_{SHV} was 80.08, while, 88.08 was the melting curve of bla_{OXA-10} . The study revealed the presence of ESBL genes in clinical strains of *K. pneumoniae* isolated from Iraqi clinical subjects. The detection of these genes was highly sensitive by RT-PCR than conventional PCR.

Keywords: Extended-spectrum β -lactamase, Beta-Lactam, Antibiotic sensitivity testing, UTI.

Introduction

Klebsiella pneumoniae is an essential adaptable microbiome that causes different human diseases, such as pneumonia, septicaemia, diarrhea and liver inflammations (Cao *et al.*, 2014; Guo *et al.*, 2017). It is a recognized infectious disease linked with patient illness and death (Cabral *et al.*, 2012). Moreover, *K. pneumoniae* is also associated with food borne disease (Kim *et al.*, 2015; Davis *et al.*, 2016). The creation of β -lactamases in G-ve bacteria is the significant cause of resistance to β -lactam antibiotics. Increasing numbers of antibiotic-resistant is a concern in the field of infections over the past few years. (EFSA, 2011; Pitout and Laupland, 2008; Liebana *et al.*, 2013). Extended-spectrum β -lactamases (ESBLs) are capable of hydrolyzing different types of β -lactam antimicrobial, which were evaluated by the World Health Organization as "antimicrobials of critical importance" (WHO, 2017). In addition, resistant strains regularly have one or more ESBL gene from diverse families of resistance genes. This may result in interference with the susceptibility test result. Consequently, reliable identification and characterization of producers of β -lactamase needs genotypic confirmation (EFSA, 2011). Enterobacteriaceae family reported to have more than 200 types of ESBL worldwide. *K. pneumoniae* and *Escherichia coli* are the furthestmost bacteria generating of ESBLs. Furthermore, other bacteria are rarely detected to produce ESBLs (Zaniani *et al.*, 2012). The bla_{OXA} gene was present in a wide variety of gram negative species in plasmid and integron locations. It has often been found that the bla_{OXA} gene is associated with genes that encode β -lactamases. OXA β -lactamase, like most ESBLs, substantially hydrolyzes ureidopenicillin and narrow-spectrum cephalosporin. European surveillance data showed a low incidence (less than 0.5%) of carbapenem-resistant *K. pneumoniae* in many countries of north Europe. Italy and Greece, on the other hand, had endemic frequent problems of 34.3% in Italy and 59.4% in Greece (EARSS 2013). Carbapenemase-producing *Klebsiella* is known to be

widespread elsewhere in the Northeastern United States and parts of Asia with rare reports from Australia and Africa (Chen *et al.*, 2012). The present study was aimed to detect ESBL genes (bla_{OXA-10} and bla_{SHV}) from *K. pneumoniae* isolated from Iraqi clinical subjects severing UTI infection by using conventional PCR and RT-PCR.

Materials and Methods

In this study, 19 strains *K. pneumoniae* isolated from urinary tract infection (UTI) and determined to be clinically significant were collected from laboratories of Al-Nokhba diagnostics center, Baghdad, Iraq. Every one of the subjects was completely aware and taught about the present research and formal approval was signed. The urine samples were cultured on MacConkey and UTI agar (HiMedia, India). The colony morphology, shape, size, swarming phenomenon and texture of the bacteria were observed.

Conventional PCR

The DNA of the samples were extracted using wizard genomic DNA purification kit (Promega, USA) as instructed by manufacturer. The PCR amplification mixture which used for detection of genes are FIREPol® Master Mix 5X (Solis BioDyne, Europe). FIREPol® Master Mix 5X (Solis BioDyne, Europe) is the PCR amplification combination used for gene detection. The PCR combination included 4 μ l FIREPol® Master Mix 5X, 2 μ l of DNA template, 1 μ l (1 mM) of each forwarded and reversed primers and 10 μ l of nuclease free water to complete the amplification mixture to 20 μ l. The amplification was performed under certain conditions for 35 cycles: initial denaturation 95 °C for 3 min, denaturation 95 °C for 45 sec, annealing 52 °C for 30 sec, extension 72 °C for 50 sec, final extension 72 °C 10 min. The forward primer of bla_{SHV} (5'-CGCCTGTGTATTATCTCCCT-3') and the reverse primer of bla_{SHV} (5'-CGAGTAGTCCACCAGATCCT-3') while the forward primer of bla_{OXA-10} is (5'-GTCTTCGAGTACGGCATT-3') and the reverse primer of bla_{OXA-10} is (5'-ATTTTCTTAGCGGCAACTTAC-3'). The

products obtained from PCR amplification was evaluated by gel electrophoresis.

Real time PCR

The real-time PCR (RT-PCR) was employed to detect bla_{SHV} and bla_{OXA-10} genes from *K. pneumoniae* by 5x HOT FIREPol® EvaGreen® qPCR Supermix (Solis BioDyne, Europe). The forward primer of bla_{SHV} (5'-TCCCATGATGAGCACCTTTAAA-3') and the reverse primer of bla_{SHV} (5'-TCCTGCTGGCGATAGTGGAT-3') while the forward primer of bla_{OXA-10} is (5'-GTCTTTCGAGTACGGCATT-3') and the reverse primer of bla_{OXA-10} is (5'-ATTTCTTAGCGGCACTTAC-3'). The amplicons were detected and confirmed by using dye EvaGreen (Invitrogen, Eugene, OR) and melt curve analysis. The final mixture of RT-PCR was 20 μ l included 5x HOT FIREPol® EvaGreen® qPCR Supermix 4 μ l, both forward and reverse primer (10 pmol/ μ l) 0.55 μ l, DNA template 2 μ l and H₂O PCR grade for up to 20 μ l. The 40 cycle of RT-PCR performed under the following conditions: initial activation 95°C for 12 min, denaturation 95°C for 15 sec, annealing 60°C for 30 sec, elongation 72°C for 20-30 s and melt curve analysis 65-95°C at 0.2°/sec. The melting curve was obtained at the wavelength of 475/520 nm

Results

Of 19 different strains of *K. pneumoniae*, 15 and 16 strains found to harbor bla_{SHV} and bla_{OXA-10} genes, respectively. Fig. 1 and Fig. 2 shows the bands of the conventional PCR products of bla_{SHV} and bla_{OXA-10} genes. The RT-PCR analysis assay was determined and distinguished by its specific T_m value. The bla_{OXA-10} and bla_{SHV} genes were detected in all the strains of *K. pneumoniae* at melting curve 88.08 and 80.08, respectively. Fig. 3 and Fig. 4 exhibited the melt curve of β -lactamase genes (bla_{OXA-10} and bla_{SHV}) detected from *K. pneumoniae*, respectively.

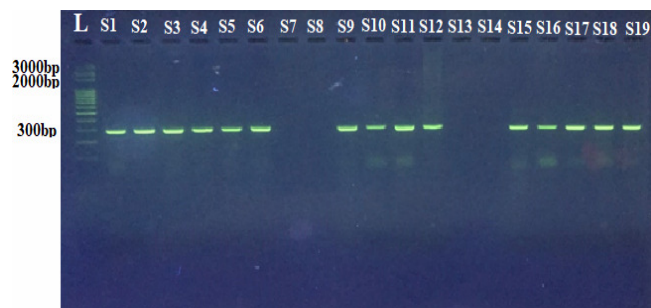


Fig. 1: PCR products of bla_{SHV} primer from different strains of *K. pneumoniae*. L: DNA ladder.

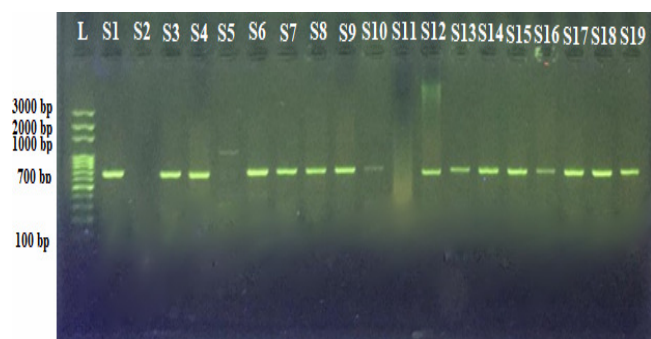


Fig. 2: PCR products of bla_{OXA-10} primer from different strains of *K. pneumoniae*. L: DNA ladder.

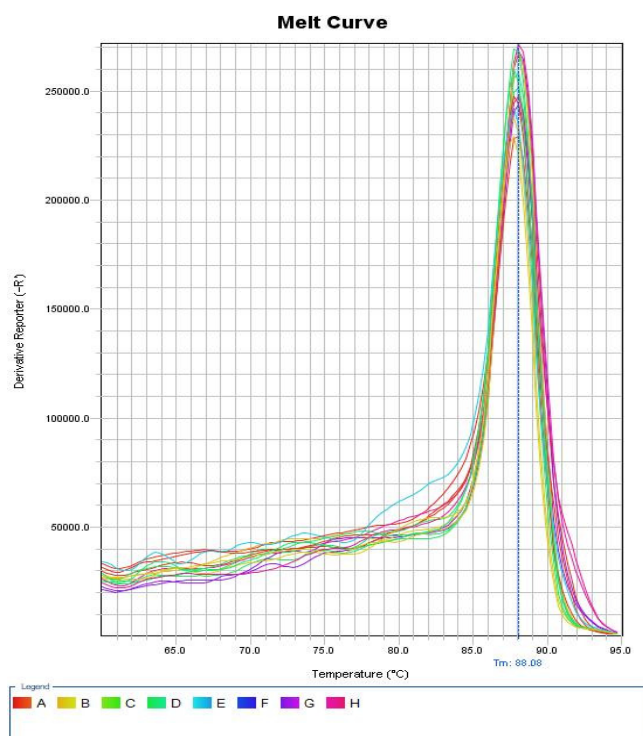


Fig. 3: Melting curve of bla_{OXA-10} from *K. pneumoniae*

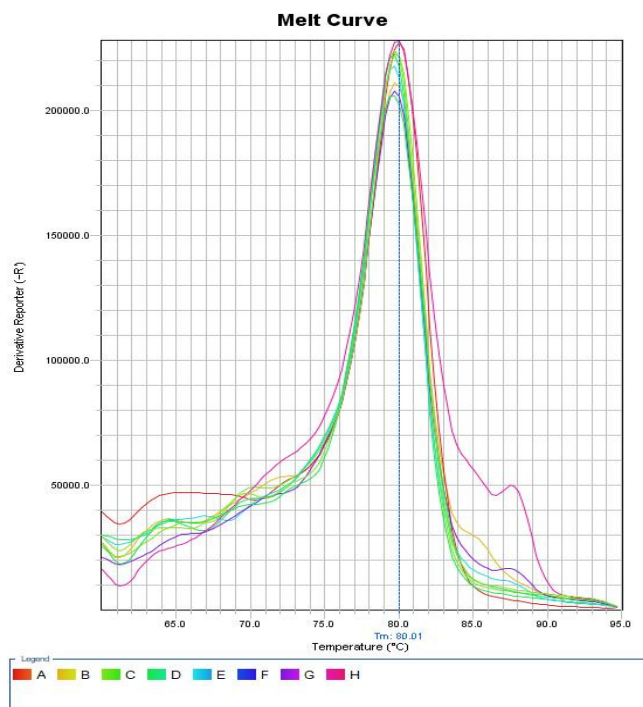


Fig. 4: Melting curve of bla_{SHV} from *K. pneumoniae*

Discussion

The treatment by β -lactam antibiotics against microorganisms may fail due to the expression of ESBL genes. The bacteria that produce β -lactamase particularly Enterobacteriaceae is considered as developing problem worldwide (Liebana *et al.*, 2013; Pitout and Laupland, 2008). The fast and accurate identification of these bacteria is of quite important to control the spread of these resistant bacteria.

In the present study, we detected bla_{SHV} and bla_{OXA-10} genes from different strains of *K. pneumoniae* isolated from

clinical subjects. This is lead to fact that these isolates were resistant to β -lactam antibiotics, as bla_{SHV} and bla_{OXA-10} genes are encoding to β -lactamase (Abrar *et al.*, 2019). This outcome considered as one of very few reports demonstrate the ESBL genes among Iraqi clinical strains of *K. pneumoniae*. This high prevalence of resistant is troublesome as few antibiotics are existing for treatment. The bla_{SHV} gene was mostly detected from *Escherichia coli* and *Klebsiella* sp., while bla_{OXA} gene primarily found in *Acinetobacter* sp. and *Pseudomonas* sp (Page, 2008; Sharma *et al.*, 2013; Abrar *et al.*, 2018). Recent studies found that the prevalence of ESBLs is high in strains of *K. pneumoniae* (Bajpai *et al.*, 2017; Zaniani *et al.*, 2012). In other hand, *E. coli* was found to have more ESBL genes than *Klebsiella* sp. and *Enterobacter* sp. (Bari *et al.*, 2015; Abrar *et al.*, 2019). The current report indicated two β -lactamase genes within the same *K. pneumoniae* strains. This finding was in accordance with previous reports (Bajpai *et al.*, 2017). The contrasts between our investigations with other researchers have shown the predominance of ESBL can change among the respective area.

In Iraq, due to shortage of surveillance reports, insufficient contagion control organization, incorrect clinical diagnosis and deficiency of modern laboratory equipment led for the appearance of resistant bacteria to antibiotics. That is in agreement with reports of Asian countries which described to be extremely affected by multidrug resistant bacteria (Abrar *et al.*, 2017; Jean and Hsueh *et al.*, 2011; Hsueh *et al.*, 2011).

The conventional PCR detected bla_{SHV} and bla_{OXA-10} genes from 15 and 16 strains of *K. pneumoniae*, respectively (Fig. 1 and Fig. 2). Not all the strains demonstrated bands for bla_{SHV} and bla_{OXA-10}. That's maybe because the absence of the genes in these bacterial strains or the presence of other genes of ESBL. However, the detection of the same genes was more sensitive and accurate by RT-PCR (Fig. 3 and Fig. 4). The results indicated that the conventional PCR shows a solid relationship with the RT-PCR while represent the equivalent sensitivity and specificity. Conventional PCR allowed the detection of critical and anaerobic microorganisms, yet RT-PCR designated a significant predominance in the identification of microbial infections. This results are found to be similar with other reports which stated that the RT-PCR is highly sensitive molecular technique and the bacterial contaminations is very low (Morel *et al.*, 2015; Chen *et al.*, 2018).

Conclusion

The outcome of the present investigation will give valuable knowledge about ESBL genes produced by *K. pneumoniae* of Iraqi clinical isolates. To understand the resistance process and the approach to developing new antimicrobials. The RT-PCR is highly sensitive for detection of different genes, hence, could be used constantly in research centers for medical microbiology to diagnose ESBL genes.

References

- Abrar, S.; Ain, N.U.; Liaqat, H.; Hussain, S.; Rasheed, F. and Riaz, S. (2019). Distribution of bla CTX– M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum- β -lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. *Antimicrobial Resistance and Infection Control*, 8(1): 80.
- Abrar, S.; Hussain, S.; Khan, R.A.; Ain, N.U.; Haider, H. and Riaz, S. (2018). Prevalence of extended-spectrum- β -lactamase-producing Enterobacteriaceae: first systematic meta-analysis report from Pakistan. *Antimicrobial Resistance and Infection Control*, 7(1): 26.
- Abrar, S.; Vajeeha, A.; Ul-Ain, N. and Riaz, S. (2017). Distribution of CTX-M group I and group III β -lactamases produced by *Escherichia coli* and *Klebsiella pneumoniae* in Lahore, Pakistan. *Microbial pathogenesis*, 103: 8-12.
- Bajpai, T.; Pandey, M.; Varma, M. and Bhatambare, G.S. (2017). Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. *Avicenna journal of medicine*, 7(1): 12.
- Bari, F.; Shah, H. and Wazir, R. (2015). Frequency and Detection of Extended Spectrum Betalactamase in *Escherichia coli* and *Klebsiella pneumoniae*: A Study At Lady Reading Hospital Peshawar. *Journal of Postgraduate Medical Institute (Peshawar-Pakistan)*, 29(4).
- Cabral, A.B.; Melo, R.D.C.D.A.; Maciel, M.A.V. and Lopes, A.C.S. (2012). Multidrug resistance genes, including blaKPC and blaCTX-M-2, among *Klebsiella pneumoniae* isolated in Recife, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 45(5): 572-578.
- Cao, X.; Xu, X.; Zhang, Z.; Shen, H.; Chen, J. and Zhang, K. (2014). Molecular characterization of clinical multidrug-resistant *Klebsiella pneumoniae* isolates. *Annals of clinical microbiology and antimicrobials*, 13(1): 16.
- Chen, G.H.; Tang, X.Y.; Sun, Y.; Zhou, L.; Li, D.; Bai, Y. and Ma, J.Y. (2018). Development of a SYBR green-based real-time quantitative PCR assay to detect PCV3 in pigs. *Journal of virological methods*, 251: 129-132.
- Chen, L.F.; Anderson, D.J. and Paterson, D.L. (2012). Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance. *Infection and drug resistance*, 5: 133.
- Davis, G.S. and Price, L.B. (2016). Recent research examining links among *Klebsiella pneumoniae* from food, food animals, and human extra intestinal infections. *Current environmental health reports*, 3(2): 128-135.
- EFSA (2011). Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β -lactamases and/or AmpC b-lactamases in food and food-producing animals. *EFSA Journal.*, 9: 2322.
- European Antimicrobial Resistance Surveillance System (EARSS). (2013). EARSS annual report. European Centre for Disease Prevention and Control.
- Guo, Y.; Wang, S.; Zhan, L.; Jin, Y.; Duan, J.; Hao, Z. and Wang, L. (2017). Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. *Frontiers in cellular and infection microbiology*, 7: 24.
- Hsueh, P.R.; Hoban, D.J.; Carmeli, Y.; Chen, S.Y.; Desikan, S.; Alejandria, M. and Binh, T.Q. (2011). Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract

- infections in Asia-Pacific region. *Journal of infection*, 63(2): 114-123.
- Jean, S.S. and Hsueh, P.R. (2011). High burden of antimicrobial resistance in Asia. *International journal of antimicrobial agents*, 37(4): 291-295.
- Kim, H.S.; Chon, J.W.; Kim, Y.J.; Kim, D.H.; Kim and M.S., Seo K.H. (2015). Prevalence and characterization of extended-spectrum-β-lactamase-producing *Escherichia coli*, and *Klebsiella pneumoniae*, in ready-to-eat vegetables. *Int J Food Microbiol.*, 207: 83-86.
- Liebana, E.; Carattoli, A.; Coque, T.M.; Hasman, H.; Magiorakos, A.P.; Mevius, D. and Torren-Edo, J. (2013). Public health risks of enterobacterial isolates producing extended-spectrum β-lactamases or AmpC β-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. *Clinical infectious diseases*, 56(7): 1030-1037.
- Morel, A.S.; Dubourg, G.; Prudent, E.; Edouard, S.; Gouriet, F.; Casalta, J.P. and Raoult, D. (2015). Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. *European Journal of Clinical Microbiology and Infectious Diseases*, 34(3): 561-570.
- Page, M.G.P. (2008). Extended-spectrum β-lactamases: structure and kinetic mechanism. *Clinical Microbiology and Infection*, 14: 63-74.
- Pitout, J.D. and Laupland, K.B. (2008). Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *The Lancet infectious diseases*, 8(3): 159-166.
- Sharma, M.; Pathak, S. and Srivastava, P. (2013). Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. *Journal of clinical and diagnostic research: J Clin Diagn Res*, 7(10): 2173.
- World Health Organization. (2017). Critically important antimicrobials for human medicine: ranking of antimicrobial agents for risk management of antimicrobial resistance due to non-human use.
- Zaniani, F.R.; Meshkat, Z.; Nasab, M.N.; Khaje-Karamadini, M.; Ghazvini, K.; Rezaee, A. and Hoseini, M.D. (2012). The prevalence of TEM and SHV genes among extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*. *Iranian journal of basic medical sciences*, 15(1): 654.