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

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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 microbe that causes different human diseases, such as pneumonia, septicemia, 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. pneumonia 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 furthermore 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 uredopenicilllin 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 μl FIREPol® Master Mix 5X, 2 μl of DNA template, 1 μl (1 mM) of each forwarded and reversed primers and 10 μl of nuclease free water to complete the amplification mixture to 20 μl. 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'- CGCGTGTAGTTATATCTCCCT-3') and the reverse primer of bla_{SHV} (5'-CGAGTAGTCCACCAGATCCT-3') while the forward primer of bla_{OXA-10} is (5'-GTCCTTTCAGCTACGGCATTA-3') and the reverse primer of bla_{OXA-10} is (5'-ATTICTTAGCGGCACCTAC-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\textsubscript{SHV} and bla\textsubscript{OXA-10} genes from \textit{K. pneumoniae} by 5x HOT FIREPol\textsuperscript{®} EvaGreen\textsuperscript{®} qPCR Supermix (Solis BioDyne, Europe). The forward primer of bla\textsubscript{SHV} (5'-TCCCATGATGAGCACCTTTAAA-3') and the reverse primer of bla\textsubscript{SHV} (5'-TCCTGCTGGCGATAGTGGAT-3') while the forward primer of bla\textsubscript{OXA-10} is (5'- GTCTTTGATCGTACCATTAC-3') and the reverse primer of bla\textsubscript{OXA-10} is (5'- ATTTTCTTAGCGCAACTTAC-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 µl included 5x HOT FIREPol\textsuperscript{®} EvaGreen\textsuperscript{®} qPCR Supermix 4 µl, both forward and reverse primer (10 pmol/µl) 0.55 µl, DNA template 2 µl and H\textsubscript{2}O PCR grade for up to 20 µl. 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 \textit{K. pneumoniae}, 15 and 16 strains found to harbor bla\textsubscript{SHV} and bla\textsubscript{OXA-10} genes, respectively. Fig. 1 and Fig. 2 shows the bands of the conventional PCR products of bla\textsubscript{SHV} and bla\textsubscript{OXA-10} genes. The RT-PCR analysis assay was determined and distinguished by its specific Tm value. The bla\textsubscript{OXA-10} and bla\textsubscript{SHV} genes were detected in all the strains of \textit{K. pneumoniae} at melting curve 88.08 and 80.08, respectively. Fig. 3 and Fig. 4 exhibited the melt curve of \(\beta\)-lactamase genes (bla\textsubscript{OXA-10} and bla\textsubscript{SHV}) detected from \textit{K. pneumoniae}, respectively.

![Fig. 1: PCR products of bla\textsubscript{SHV} primer from different strains of \textit{K. pneumoniae}. L: DNA ladder.](image1)

![Fig. 2: PCR products of bla\textsubscript{OXA-10} primer from different strains of \textit{K. pneumoniae}. L: DNA ladder.](image2)

![Fig. 3: Melting curve of bla\textsubscript{OXA-10} from \textit{K. pneumoniae}](image3)

![Fig. 4: Melting curve of bla\textsubscript{SHV} from \textit{K. pneumoniae}](image4)

**Discussion**

The treatment by \(\beta\)-lactam antibiotics against microorganisms may fail due to the expression of ESBL genes. The bacteria that produce \(\beta\)-lactamase particularly Enterobacteriaceae is considered as developing problem worldwide (Liebana \textit{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\textsubscript{SHV} and bla\textsubscript{OXA-10} genes from different strains of \textit{K. pneumoniae} isolated from
clinical subjects. This is to lead to fact that these isolates were resistant to β-lactam antibiotics, as blaSHV and blaOXA-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 blaSHV gene was mostly detected from *Enterobacteria coli* and *Klebsiella sp.*. while blaOXA 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. pneumonia 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, 2011; Abrams et al., 2015; Chen, 2012). In other hand, *E. coli* was found to have more ESBL genes than *Klebsiella* sp. and *Enterobacter* sp. (Page, 2008; Sharma et al., 2013; Abrar et al., 2018). Recent studies found that the prevalence of ESBL 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. pneumonia 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.

The conventional PCR detected blaSHV and blaOXA-10 genes from 15 and 16 strains of *K. pneumoniae*, respectively (Fig. 1 and Fig. 2). Not all the strains demonstrated bands for blaSHV and blaOXA-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. These 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**


