IDENTIFICATION AND PHYLOGENETIC STUDY OF Klebsiella pneumoniae VIA MOLECULAR-BASED TECHNIQUES TARGETING 16S rRNA, magA, and rmpA IN CAMELS FROM AL-DIWANIYAH CITY, IRAQ

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ABSTRACT

This study was as an investigation to identify the presence of Klebsiella pneumoniae in the livers and lungs of camels in Al-diwaniyah City, Iraq. Here, 50 specimens were sampled from each organ of 50 camels. In the beginning, regular cultivation and biochemical tests were performed for primary detection. Then, a multiplex polymerase chain reaction (mPCR) targeting specific pieces of 16S rRNA, mucoviscosity-associated A (magA), and regulator of mucoid phenotype A (rmpA) genes was done for more confirmative identification of the bacterium. After that, the PCR-based products of the 16S rRNA gene were subjected to partial sequencing in which the results were used to generate a phylogenetic tree to compare our isolates with some previously identified strains of the K. pneumoniae. The results of the primary detection showed the presence of this bacterium in 30 (60%) and 42 (84%) of the liver and lung samples respectively. The mPCR results revealed the amplification of the tested genes at 30(100%)16S rRNA, 25 (83%) magA, and 0 (0%) rmpA in the liver samples and 42(100%) 16S rRNA, 35 (92%) magA, and 30 (78.9%) rmpA in the samples of lungs. The sequencing results identified 2 isolates (MH119051.1) and (MH119052.1) which gave close matching with some global stains such as KM203760.1, an Indian isolate. Our study gives valued information about the presence of this bacterium that causes infection to the camels in the studied city.

Keywords: Klebsiella pneumoniae, magA, PCR, rmpA, sequencing, 16S rRNA


INTRODUCTION

The major health problems caused by Klebsiella pneumoniae are considered as a common in human and animals. These infections sometimes may arise from contaminated places such as hospitals. These bacteria are appreciated to be among many opportunistic microorganisms 1,2. The bacterium causes a liver-abscess
condition as that was reported in different studies from Asia, pacific, and USA places. In relation to resistance to immunity of human or animals, mucoviscosity-associated gene A (magA) gene was detected to increase the capabilities of this bacterium to immunity represented by antibodies and phagocytosis, and it is also reflects the bacterial behavior in increasing mucoviscosity. Liver-abscess induction was also linked to the virulence that is generated in K. pneumoniae via regulator of mucoid phenotype A (rmpA) gene. Camels in various regions of the world and especially South of Iraq are considered as important food animals for the meat and the milk they produce. Camels could suffer many disease conditions, respiratory infections could play important roles in morbidities and mortalities in camels. Camels play important roles in spreading the disease caused by K. pneumoniae to other animals and humans to be as a big part of human-respiratory infection sources. To induce respiratory infection, K. pneumoniae relies on camel health conditions and the environment that these camels live in such as stress conditions affecting the animals and contaminated habitats. Little or absent information is known about the presence of K. pneumoniae in camels from Al-Diwaniyah City, Iraq. For this reason, the present study was intended to identify the presence of Klebsiella pneumoniae in the livers and lungs of camels in Al-diwaniyah City, Iraq.

MATERIALS AND METHODS

Sampling and cultivation

This study was as an investigation to identify the presence of Klebsiella pneumoniae in the livers and lungs of camels in Al-diwaniyah City, Iraq. Here, 50 specimens were sampled from each organ of 50 camels. In the beginning, regular cultivation and biochemical tests were performed for primary detection. These samples were cultivated on macConkey and blood agars. The cultivation processes were performed according to. The cultivation was activated using CHROMagar Orientation. The primary detection via morphological and biochemical feature identification was made according to.

DNA extraction

A DNA extraction kit (Geneaid, USA) was used to perform the process of extracting the DNA from fresh growth of Klebsiella pneumoniae. The procedure was generated according to the manufacturer’s protocol. The DNA was measured for quality and quantity using a NanoDrop.

Multiplex-PCR of K. pneumoniae

The primers used to amplify regions in the 16S rRNA gene were designed using NCBI- and Plus3-based tool and were deposited as LT599801.1 in the NCBI-GenBank. For the magA, and rmpA genes, the primers were used from. These primers are shown in table 1. The reaction of the mPCR employed 3μl DNA, 0.7μl (10pmol) for each primer, 12.5μl of mastermix (2x) of (1.5mM MgCl2, 1U Taqpolymerase, and 200μM dNTPs), and 11.4μl H2O for molecular biology use (Bioneer, Korea). The conditions of the thermocycler were 1 cycle at 94°C for 5min of initial denaturation, 35 cycles (denaturation at 94°C for 30s, annealing at 60°C for 1.5min, and extension at 72°C for 1.5min), and final extension at 72°C for 10min. Using 2%-agarose gel including 0.5μg/ml ethidium bromide, DNA bands were separated via electrophoresis and visualized via a UV imager.
Table 1: The primers of the study

<table>
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<th>Primer</th>
<th>Sequence</th>
<th>Product size(bp)</th>
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<tr>
<td>KP-16S rRNA,</td>
<td>F</td>
<td>GGAAGTGAACAGCGGTCCAG</td>
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<td>strain K-18</td>
<td>R</td>
<td>CCAGGTAAGGTTCTTCGCT</td>
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<tr>
<td>magA</td>
<td>F</td>
<td>GGTGCTCTTACATTGCAC</td>
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<tr>
<td></td>
<td>R</td>
<td>GCAATGGCATTGCTGTTAGC</td>
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<tr>
<td>rmpA</td>
<td>F</td>
<td>ACTGGCTCATGCTTCA</td>
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<td></td>
<td>R</td>
<td>CTTCAGTGACATTTTCA</td>
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DNA sequencing of the PCR product

The 16S rRNA-based PCR products were sent out to do sequencing at Bioneer Company, Korea, using AB DNA sequencing system. The resulted sequences were used to compute the phylogenetic tree using MEGA 6.0 software and after NCBI-Database-based searching processes were performed. The tree was generated relying on the Neighbor-Joining method. The distances were computed via the Maximum Composite Likelihood method.\(^{15,16}\)

RESULTS

Isolation and identification

*K. pneumonia* colonies were purple on EMB, yellow slant/yellow and no H\(_2\)S in triple sugar iron (TSI), pink on MacConky agar, and metallic-blue large-rounded colonies on orientation Chromeagar, figure 1. Table 2 shows the biochemical test for this bacterium.

![Figure 1: Metallic-blue rounded-large colonies of *Klebsiella pneumoniae* on orientation chrome agar.](image)

Table 2: Biochemical tests of *K. pneumonia*.

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Multiplex-PCR
The mPCR results revealed the amplification of the tested genes at 30(100%) 16S rRNA, 25 (83%) magA, and 0 (0%) rmpA in the liver samples and 42(100%) 16S rRNA, 35 (92%) magA, and 30 (78.9%) rmpA in the samples of lungs, figure 2 and figure 3.

Figure 2: Agarose-gel image of 16S rRNA, 770bp, gene amplification. M is the ladder, 10000-500bp. Lanes,1-4, are the positive amplifications for this gene.

Figure 3: Agarose-gel image of magA, 1283bp, and rmpA, 536bp, gene amplification. M is the ladder, 1500-300bp. Lanes,1-4, are the positive amplifications for these genes

16SrRNA gene partial sequencing
The results of the sequencing revealed 2 isolates (MH119051.1) and (MH119052.1) which gave close matching with some global stains such as KM203760.1, an Indian isolate, figure 4.

Figure 4: Phylogenetic tree of the current study isolates compared to global strains of *K. pneumoniae*. The tree was generated relying on the Neighbor-Joining method. The distances were computed via the Maximum Composite Likelihood method.

DISCUSSION

*Klebsiella pneumoniae* causes respiratory infection in human and animals. These bacteria are opportunistic microorganisms, and they may induce nosocomial infections that arise from contaminated places. Camels could be a good source for respiratory infections in humans. The results of this study indicate that this bacterium was present in the livers and lungs of the tested camels, as they were proven by the results of the primary tests. These results agree with 17 who isolated *Klebsiella pneumoniae* at 9.1% from normal lungs of camels from Ethiopia. Our current results also agree with 18 who detected the presence of *Klebsiella pneumoniae* at 26.71% inducing a subclinical infection in lungs of camels from Cairo, Egypt. In Sudan, the bacterium was isolated from lungs of infected camels 18, and their results agree with current study results. Although these studies were positive regarding the detection of *Klebsiella pneumoniae* in the lungs of normal and infected camels, they lack the use of molecular-based techniques to identify this bacterium and its virulent genes in the tissues of lungs. Here, we employed the use of mPCR to detect this bacterium and its...
related virulent genes in livers and lungs of camels. The results of mPCR showed the amplification of these tested genes at 30(100%) 16S rRNA, 25 (83%) magA, and 0 (0%) rpmA in the liver samples and 42(100%) 16S rRNA, 35 (92%) magA, and 30 (78.9%) rpmA in the samples of lungs. These results agree with who detected hypermucoviscous K. pneumoniae isolates from infected lungs of camels using a conventional-PCR technique that targeted magA gene. This indicates that our isolates are from the virulent strains of K. pneumoniae since we already detected this gene using mPCR on samples from livers and lungs of camels. To extend the limits of previous studies, sequencing was an interesting part of our study. The results unveiled the presence of 2 isolates (MH119051.1) and (MH119052.1) which gave close matching with some global stains such as KM203760.1, an Indian isolate. This indicates strong nucleotides matching with our isolates, and it also refers that our isolates and these global strains might have descended from the same direct ancestor.

CONCLUSION

Our study gives valued information about the presence of this bacterium that causes infection to the camels in the studied city.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING: Self-funding

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