Molecular Detection Of Klebsiella Pneumoniae Isolated From Different Clinical Source

Boraq Abbas Abd Alwahed, Noor Saad latteef, Wathiq Abbas Hatite Al-Daraghi*

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.
Corresponding Author: wathiqaldrghi@yahoo.com

ABSTRACT
The World Health Organization has listed Klebsiella pneumoniae as one of the global priority pathogens in critical need of next-generation antibiotics. Compared to other Gram-negative pathogens, K. pneumoniae accumulates a greater diversity of antimicrobial-resistant genes at a higher frequency. This study included collecting 60 samples from different sources (wounds, Burns, sputum, urine), and the samples were diagnosed by traditional methods, culturing samples on different media, then conducting biochemical tests, and initial tests showed that 50% of the samples were Klebsiella pneumoniae bacteria, and the rest for species (Escherichia coli 30%, Streptococcus pneumoniae 10%, Pseudomonas aeruginosa 5%, Staphylococcus aureus 5%), then Klebsiella pneumoniae was diagnosed by polymerase chain reaction (PCR) using the diagnostic gene (infB). The results showed that only 15 samples out of 30 had this gene. It was diagnosed as Klebsiella pneumoniae, after that CTX resistance gene was examined, and the results showed the presence of this gene in 67% isolates and its absence in 33%. The results showed the presence of this gene in bacteria isolated from urinary tract infection, and with a small percentage of samples isolated from

INTRODUCTION
Klebsiella spp. are the causative agent of numerous kinds of infections in humans including: urinary tract infections, respiratory tract infections, and blood stream infections. It is involved in extra-intestinal infections including urinary tract infections, cystitis, pneumoniae, surgical wound infections and life-threatening infections, such as endocarditis and septicemia. It is also an important cause of serious community onset infections such as necrotizing pneumonia, pyogenic liver sores and endogenous endophthalmitis. Klebsiella species (spp.) are Gram-negative, non-motile, rod shape, and soil and readily isolated from mammalian mucosal surfaces belong to the Enterobacteriaceae family. Klebsiella spp. are generally found in animal and human gut microbiota. They colonize a wide range of hosts including plants and mammals and can grow ubiquitously in water and soil and Klebsiella spp. are generally opportunistic pathogens and do not usually affect healthy individuals (Wyres and Holt, 2018). Klebsiella spp. utilize the following virulence traits to protect themselves from the host immune response capsular polysaccharides (CPS), lipopolysaccharides (LPS), siderophores, fimbriae (alternatively, pil), a type VI secretion system, outer-membrane proteins, porins, efflux pumps, an iron transport system, biofilms, and allantoin metabolism. Among these, CPS, LPS, siderophores, and fimbriae are well characterized virulence factors of Klebsiella spp. These virulence factors assist Klebsiella spp. in evading the innate immune response of the host and to survive in different sites within the host, rather than actively suppressing host immune system components (Domenico et al., 1994; Hsieh et al., 2019). This report aims to study the characters and virulence factors of Klebsiella pneumoniae and Screening of the CTX resistance gene from different clinical sources.

Sample Collection: During the period extended From March 2020 to July 2020.60 samples were collected from Baghdad city, these samples included: wound swab (11), urine (25), sputum (16) and burns (8)

Bacterial Isolation and Identification: Bacterial isolates are diagnosed by conventional microbiological methods (colonial morphology, Gram staining and biochemical tests) according to (Cheesebrough, 1990), and molecular method (PCR).

PCR amplification: DNA template of all isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted for the infB 1 and gene using the primers listed in Table 1 and for the CTX using the primers listed in Table 1.

A reaction mixture (25 μl) contained 2 μl of DNA, 1 μl of each primer, 12.5 μl of Master Mix 2Xand 8.5 μl of Nuclease Free Water. The experiment was continued according to the following program: initial denaturation at 95°C for 5 minutes, followed by 30 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 7 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye and visualized by Gel Doc apparatus (BioRad, USA) (Table 2).

Table 2: PCR program

<table>
<thead>
<tr>
<th>Steps</th>
<th>°C</th>
<th>m: s</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95</td>
<td>05:00</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>00:30</td>
<td></td>
</tr>
</tbody>
</table>
Table 1: Primers used in this study

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Seq.</th>
<th>Annealing Temp. (°C)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>infB1-F</td>
<td>5'-CTCGCTGCTGGACTATATTCG-3'</td>
<td>55</td>
<td>462</td>
</tr>
<tr>
<td>infB1-R</td>
<td>5'-CGCTTTCAGCTCAAGAACTTC-3'</td>
<td>55</td>
<td>462</td>
</tr>
<tr>
<td>CTX-F</td>
<td>5'-GACGATGTCACTGGCTGAGC-3'</td>
<td>60</td>
<td>499</td>
</tr>
<tr>
<td>CTX-R</td>
<td>5'-AGCCGCCGACGCTAATAC-3'</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

60 samples were collected and diagnosed in different methods; the results showed that 50% of the samples were for *Klebsiella pneumoniae* distributed, the largest percentage was isolated from urine as the following (43% of urine, 27% of sputum, 20 of wounds, 10% of burns) and the remaining 50% The different bacterial species were (*Escherichia coli* 30%, *Streptococcus pneumoniae* 10%, *Pseudomonas aeruginosa* 5%, *Staphylococcus aureus* 5%). The samples were then subjected to different types of media and biochemical tests to isolate the bacteria (fig. 1).

Figure (1): The percentage of presence of *K.pneumonia* in different sources
**Biochemical test** Results in table (3) showed that the isolates gave positive results for catalase due to the ability to produce catalase enzyme (that reduce hydrogen peroxide to water and oxygen gas bubbles), negative results for oxidase due to the inability to produce cytochrome C oxidase (that oxidase tetramethyl-p phenylenediamine), positive results for Citrate utilization due to the citrate considered a sole carbon source. All isolates were negative for indole testing and here is an important point to distinguish between K.pneumonia and klebsiella oxytoca.

**Table (3)** morphological characterization, gram staining and biochemical tests results of K.pneumonia

**Genomic DNA Extraction:**

DNA was extracted from all bacterial isolates by using rapid bacterial genomic DNA isolation kit (Promega, USA). Extraction results were good for concentration and purity which is determined by using nanodrop spectrophotometer at 260/280 nm. Concentration values of DNA ranged between 200-600 ng/μl while purity ranged from 1.45-2.1 (appendix 2) then subjected to gel electrophoresis in 1.5% agarose, the DNA bands were visible under UV as shown in the figure (2).

**Molecular Detection of InFB gene in Klebsiella pneumoniae:**

Fig. (2) Showed the result of the polymerase chain reaction (PCR) method to Detect Klebsiella pneumoniae using the InFB gene showed that only 50% of The isolates diagnosed by conventional and biochemical methods possess this gene.

**Molecular Detection of CTX genes in K. pneumoniae**

The current study showed that 67% of the samples diagnosed by Molecular methods possessed the CTX resistance gene, most of which came from patients with urinary tract infection, while the isolates that did not have this gene were from other sources. This result agree with Elif et al., (2010) they reported that CTX-M type ESBL were observed in 22.72% of E. coli isolates and frequently found in Klebsiella pneumoniae isolates.

**Figure(3)** Results of the amplification of CTX gene of Klebsiella pneumoniae bacterial species were fractionated on 1.5% agarose gel electrophoresis stain with Eth.Br. M: 100bp
REFERENCES