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Molecular Analysis of Klebsiella Pneumoniae Isolates Collected from Sputum Samples in Duhok Iraq

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Abstract

Klebsiella is an important human pathogen that may lead to both community-acquired infections and nosocomial infection. A major threat to public health is the increasing frequency of Klebsiella pneumoniae strains that are resistant to drugs. This study aimed to determine the frequency of K. pneumoniae in sputum samples obtained from patients at the Azadi Teaching Hospital in Duhok City, Iraq, as well as the 16S rRNA and rpoB genes for molecular analysis of K. pneumoniae. Eighty-three sputum samples were collected from patients at the Azadi Teaching Hospital in Duhok, Iraq, between January and May of 2024. Through morphologic and biochemical investigations, the isolates were identified. Klebsiella pneumoniae isolates were isolated from 37/83 (44.6%) sputum samples. Upon molecular examination, every isolate in our investigation displayed a PCR result of 108 bp using the K. pneumonia specific primer (rpoB), and they were able to generate a 16S rRNA gene of 1500 bp amply. Finding out how often K. pneumonia infections are among patients at Azadi Teaching Hospital in Duhok City, Iraq, is a crucial goal of this study.

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1. Introduction

Upper and lower respiratory tract infections are strongly linked to morbidity and death, particularly in older persons, those with compromised immune systems, and those with a history of lung illness [1]. Sputum is the sample most commonly used to diagnose lower respiratory tract infections because it is easy to collect and does not require invasive treatment procedures [2]. Practically and financially, a sputum culture is necessary for the diagnosis of lower respiratory tract infections. However, there's a chance that saliva and oral bacteria contaminated the sample during collection, which might compromise its validity. Additionally, this helps save time and money by preventing needless processing of contaminated samples that are unfit for use in clinical treatment [3]. Klebsiella pneumoniae is one of the most important members of the genus Klebsiella within the family Enterobacteriaceae, responsible for causing pneumonia [4]. K.lebsiella pneumoniae is the main type of Klebsiella that causes pneumonia, while K. oxytoca occurs in smaller amounts. These bacteria are Gram-negative bacilli, measuring 0.3-1 µm wide and 0.6-6 µm long, occurring singly, in pairs or short chains, and having a visible polysaccharide capsule [5]. Klebsiella colonies on MacConkey agar exhibit a large, sticky, red appearance, with a diffuse red hue indicative of glucose fermentation and acid production [4]. Klebsiella species are found naturally in the digestive tracts of both humans and animals [6], and can also cause meningitis, bronchitis, bacteriemia, and urinary tract infections [7, 8]. This infection is common, especially among people with weakened immune systems and chronic lung disease. Klebsiella species generally live in soil, water, and plant environments [4]. K.lebsiella pneumoniae is a human bacterial infection that is increasingly difficult to treat, according to a 2014 study [9]. It can lead to community- or hospital-acquired infections, such as pyogenic liver abscesses, pneumonia, and urinary tract infections. It is also associated with high rates of antibiotic resistance [10]. *16S RNA* gene sequences are now the most widely used molecular marker to classify bacterial isolates for

several reasons, including that most bacteria carry the *16S rRNA* gene, which typically forms a multigene family. Its function has not changed over time, suggesting that random changes in sequence are a more reliable measure of time (evolution). Finally, because the gene is 1,500 bases long, it can be used for informatics [11]. When studying closely related isolates, the *rpoB* gene, which encodes the beta subunit of RNA polymerase, is a great choice for bacterial identification and phylogenetic analysis. [12, 13]. In human medicine, increased antibiotic resistance in isolates of *Klebsiella* spp. A major problem worldwide. For this purpose, sputum samples were collected from patients at Azadi Teaching Hospital in Dohuk, Iraq, to identify the *16S rRNA* and *rpoB* genes for molecular analysis of *K. pneumoniae*.

2. Materials and Methods

2.1. Duration of the study:

The study was carried out between January and May of 2024.

2.2. Sample Collection: Combine the contents in a large plastic container with a screw-on cap (capacity not exceeding 100 ml) for disposal. The ages of the patients in the sample ranged between different groups, and their genders also varied. Within an hour or two, the samples were sent to the Microbiology Laboratory in the College of Nursing, Department of Basic Sciences, to perform bacterial identification and culture of the samples.

2.3. Sample Processing

2.3.1. Gram Stain:

Direct Gram stain and standard loop technique were used for sputum samples.

2.3.2. Culturing:

Sputum was cultured on various nutrient agars (nutrient, MacConkey, blood, and eosin methylene blue). Overnight, the plates were incubated at 37°C. A microscope was used to assess the Gram stain data.

2.3.3. Biochemical Tests:

A variety of tests were performed, including the INViC (Indole, Methyl red, Voges-Proskauer, and Citrate) test, the polysaccharide fermentation test, the catalase test, and the Triple Sugar Iron (TSI) test.

2.4. Klebsiella pneumonia Molecular identification:

2.4.1. DNA Extraction

In order to extract DNA, two to three fresh colonies were boiled in 800 µl of deionized water on bacterial plate. Next, a Mini Spin plus Eppendorf centrifuge was used to centrifuge the sample for 2 min at 1200 rpm in 100°C water bath. For long-term use, the crude extract containing the DNA was kept at -20°C [11].

2.4.2. Detecting DNA Purity and Concentration:

The DNA concentration and purity of each sample was measured using a NanoDrop device by the Molecules Department of the General Central Laboratory, General Directorate of Health in Dohuk. The quantity and purity of DNA were verified using a NanoDrop 2000 spectrophotometer from Thermo Scientific in Germany. DNA purity was assessed by an optical density ratio between 1.7 and 1.8, with a concentration ranging between 10 and 50 ng/µl [11].

2.4.3. Polymerase Chain Reaction (PCR):

Using the primer indicated in Table 1, the *16S rRNA* and *rpoB* genes were targeted in the DNA of *K. pneumoniae* isolates. 3 µl of DNA, 1 µl of each primer, 10 µl of Master Mix, and 5 µl of nuclease-free water were added to the reaction mixture (20 µl) [14]. The PCR machine is configured to execute many cycles at different temperatures at each step, starting with the initial denaturation stage. After the second phase (denaturation, annealing, and extension), a final additional extension is performed. Tables 2 and 3 provide an overview of the PCR protocol for *16S rRNA* and *rpoB* genes. The PCR findings were assessed using 1.5% agarose gel electrophoresis, and a gel imager was utilized to view the ethidium bromide-stained bands on the gel [11].

Table 1. Primer sequences used for PCR

Gene	Primer sequence (5'-3')	Product size (bp)	Ref.
16S rRNA-F *16S rRNA*-R	AGAGTTTGAT CCTGGCTCAG GGTTACCTTG TTACGACTT	1500	[15]
rpoB-F *rpoB*-R	CAACGGTGTG GTTACTGACG TCTACGAAGT GGCCGTTTTC	108	[13]

Table 2. Steps of PCR for *16SrRNA* gene

Steps	Temperature °C	Time m:s	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:45	35
Annealing	58	00:45	
Extension	72	00:45	
Final extension	72	07:00	1

Table 3. Steps of PCR for *rpoB* gene

Steps	Temperature °C	Time m:s	Cycle
Initial denaturation	95	05:00	1
Denaturation	94	00:30	35
Annealing	55	00:30	
Extension	72	00:30	
Final extension	72	07:00	1

2.5. Statistical analysis:
All data statistical analysis was done using SPSS version 20 (IBM USA).

3. Results and Discussion
Klebsiella pneumonia Isolation and Identification:
Gram-negative coccobacillus cells were found in the isolates studied. Based on morphological characteristics, three highly mucus strains were identified that had pink sticky colonies on McConkey medium, while pink to violet colonies appeared on eosin methylene blue medium. In conclusion, the blood medium reveals smooth, dense, sticky colonies. Among 83 sputum samples, 37 (44.6%) *Klebsiella pneumoniae* were isolated in the present study, while 46 (55.4%) showed negative results. The results were strikingly similar to the 2024 study, which found that *Klebsiella pneumoniae* (44.6%; 37/83) was the organism most frequently isolated from sputum samples, followed by *Pseudomonas aeruginosa* (35%; 29/83) and *Escherichia coli* (20.4%; 17/83). These results are also in line with another study [16] which showed that *P. aeruginosa* was isolated from 14.77% (n = 13) of samples, *E. coli* from 12.5% (n = 11) of samples, and *K. pneumoniae* from 28.40% (n = 25) of specimens, making it the most isolated organism. These findings are consistent with those of previous research [17, 18].

Molecular analysis of *Klebsiella pneumoniae* Isolates: *16SrRNA* gene

In this study, *16S rRNA* was used as a reliable marker for *K. pneumonia* species identification. Figure 1 shows how all *K. pneumonia* isolates tested gave positive results using the *16S rRNA*-based PCR detection technique.

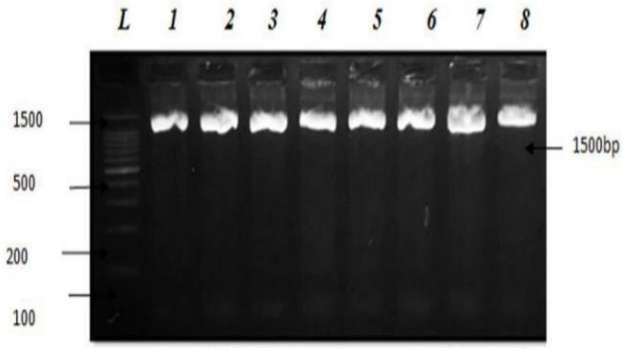

Figure 1. Represents 1.5 % agarose gel showing amplification product of *16S rRNA* gene (1500 bp); lanes 1-8 showing represent positive *K. pneumonia* isolates.

This study supports research conducted in Mosul, Iraq [15]. *16S rRNA* analysis is a suitable method for accurate identification of the isolates under investigation and offers a reliable approach to molecular analysis.

***rpoB* gene**
In this study, DNA fragments of the *rpoB* gene with a length of 108 bases were detected by gel electrophoresis in all bacterial isolates using the PCR assay (Figure 2). The high percentage of *rpoB* gene is one of the most notable findings of the study.

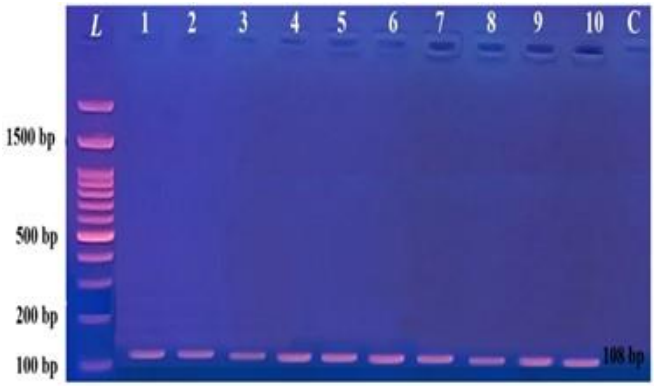

Figure 2. Represents 1.5 % agarose gel showing amplification product of *rpoB* gene (108 bp); lanes 1-10 showing represent positive *K. pneumonia* isolates.

This result is consistent with other studies from the University of Kufa and Al-Kitab University, which

showed that 100% of the PCR product represents *K. pneumonia* bacterium [13, 19]. A study conducted in Egypt in 2020 also showed that 75 samples of *Klebsiella* bacteria contain *rpo B* genes, which were identified in 74 samples (99%) of them [4]. This result is almost identical to the present results. However, it was not consistent with the results of an Iraqi study that showed that 87.93% of the PCR product represented *Klebsiella pneumoniae* [20]. This may be related to the types of laboratory identification techniques and clinical specimens used in different research.

4. Conclusions

Klebsiella pneumoniae is the source of illnesses contracted in hospitals and the general public worldwide. Finding *K. pneumonia* infections will therefore aid in reducing the mortality and morbidity rates.

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