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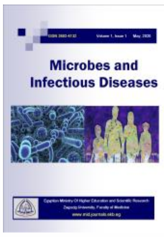

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Original article

Molecular detection of *Klebsiella pneumonia*e in urinary tract infection among pregnant women Thi Qar province

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ABSTRACT

Background: *Klebsiella pneumoniae* is a gram –negative , non-spore-forming, non motile , bacterium. These bacteria are members of the *Enterobacteriaceae* family, which is the main harmful agent of this illness during pregnancy. **Objective**: This study aims at molecular detection of *Klebsiella pneumoniae* by 16S rRNA sequencing and detection of some virulence genes and drug resistance genes, such as bla_{SHV}, *K1*, and *K2*, and identification of the genetic variations and phylogenetic tree of *K. pneumoniae*, which was isolated from the urine of pregnant women. **Methods:** In this study, we collect 150 urine samples from pregnant women. Traditional laboratory and molecular methods were used for bacterial identification, genetic variation, and phylogenetic tree of *K. pneumonia*. **Results**: recorded that the presence of about 99% homology between studied samples with *K. pneumoniae*. Seventeenth, genetic variations of 16SrRNA gene were identified in *K. pneumoniae*. Subsequently, several variations were variably distributed in the S2 and S7 samples, such as G129T, G405A, T421G, Tins652, and Ains653, while other nucleoid variations were only detected in S9, such as T403A, T960G, G970C, and A971T. Also, samples (S2 and S7) shown in current phylogenetic trees were closely related. **Conclusion**: All *Klebsiella pneumoniae* isolates were confirmed as 100% *Klebsiella pneumoniae* due to 16S rRNA. Bla_{SHV} refers to rates of 100% in all *Klebsiella pneumoniae*. *Klebsiella pneumoniae* appeared with nucleotide maturation in 16SrRN, and ten isolates were recorded with NCBI. Finally, all *Klebsiella pneumoniae* appeared to have negative results for the *K1* and *K2* genes.

Introduction

Urinary tract infections (UTIs) are a very common condition that affects people of all ages and both sexes. The prevalence of UTIs was higher in women than in males for a variety of clinical reasons, including anatomical differences, hormonal effects, behavioral patterns, and physiological and structural differences in the female urethra [1]. Bacteria, fungi , yeasts, and viruses are the microorganisms that can cause urinary tract infections [2 ,3] .The predominant pathogen responsible for urinary tract infections (UTIs) is *Escherichia coli*, accounting for 75% of all bacterial UTI cases, followed by other bacteria, including *Klebsiella* species, *Proteus, Staphylococcus aureus, Enterococcus, and Pseudomonas aeruginosa* [4].

In the *Enterobacteriaceae* family, *Klebsiella pneumoniae* is a rod-shaped, lactose-fermenting, facultatively anaerobic, oxidase-negative, encapsulated, non-motile bacterium. [5]. *K. pneumoniae* is a bacterium capable of infecting humans and causing various illness, including

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sepsis, soft tissue infections, respiratory tract infections, and urinary tract infections [6].

Materials and Methods

On hundred and fifty urine samples were sterilely collected from pregnant women suffering from UTIs at different hospitals and private clinics (AL-Shatrah Hospital, Bint AL Huda for Maternity and Children, and Al-Hussein Teaching Hospitals). The patients' ages ranged from 18 to 45 years, from September 2024 to January 2025. The clinical history of each case and full information was taking directly from the patient. All information was arranged in an informative, clear detail, such as patient name, age, pregnant month, *etc.* …

Urine samples taken midstream and stored in sterile screw-cap containers made up the specimens. Each specimen is immediately transferred under cooling conditions to the laboratory of microbiology in the Veterinary Medicine College, Al-Shatrah University, for analysis. Bacterial isolation and identification are done in the lab by culturing on MacConkey and blood agar and then by biochemical tests. The identification of the suspected *Klebsiella pneumoniae* isolates to the species level was confirmed using a molecular method.

Molecular Identification

The primers

Following the primer synthesizer company's instructions, the primers used in the interaction were dissolved in the free ddH_2O to reach a final concentration of 100 µM/µl. This stock solution was then kept at -20°C. The stock primers were used to create a working primer with a concentration of 10 µM/µl. In order to reduce freeze-thaw cycles and guarantee long-term stability, the work primer was subsequently aliquoted into smaller volumes. These aliquots were stored at -20 ºC until needed for PCR amplification experiments.

PCR condition

The extracted DNA samples were electrophoresed by combining 5µl of DNA with loading dye and loading them into wells that were subjected to an electric field (70V for 45–60 minutes) as part of the PCR procedure used to identify *Klebsiella* spp. The *K1*, *K2*, bla_{SHV}, and 16SrRNA genes' thermos cycling programs .

Results of 16S *rRNA* gene of *K. pneumoniae*

The results of the PCR technique showed the genomic DNA of all *K. pneumoniae* samples. The present results recorded that 10/10 (100%) of *K. pneumoniae* isolates gave positive results for the *16SrRNA* gene. The bands of this gene, which determined the size of the *16SrRNA* gene, nearly 1500 1500bp, are shown in Figure (1). While, *K2* genes were not detected in all samples of *K. pneumoniae*. Also, complete isolates of *K. pneumoniae* harbored the bla_{SHV} gene (100%), as in Figure (2).

Ten samples that displayed an amplicon length of roughly 1500 bp were included in the analysis of the variance of the 16S rRNA gene sequence in *K. pneumoniae.* Before sending these amplicons to sequencing, it was made sure that all the amplified amplicons had shown sharp, specific, and clean bands. The sequencing reactions indicated the confirmed identity of the amplified products by performing NCBI BLASTN. Concerning the 1500 bp PCR amplicons of the currently targeted 16S rRNA sequences, the NCBI BLASTn engine showed a high sequence similarity between the sequenced samples and *K. pneumoniae* sequences. About 99% similarity with the predicted target, which largely encompassed the coding region of the 16SrRNA gene sequences, was found by the NCBI BLASTn engine. By contrasting the recovered DNA sequences (GenBank: NZ_KQ088287.1) with the observed DNA sequences of the samples under investigation. When the 1500 bp samples were aligned with the appropriate *K. pneumoniae* referencing sequences, 17 nucleic acid differences were found (Fig. 3). These sequences were created by matching the samples under investigation to the most related sequences that have been added to the NCBI database (GenBank accession number NZ_KQ088287.1).

Figure.3. Ten bacterial samples' nucleic acid sequences were aligned with the reference 16S rRNA sequences found in the genomic DNA sequences of *K. pneumoniae*. "S" stands for sample numbers, while "ref" stands for the NCBI reference sequences. The precise locations of the detected alterations were detailed in Table 2 to provide a summary of all the findings derived from the 1500 bp segments that were sequenced.

Figure (4) showed In the inference of this phylogenetic tree, the most relative sequences to the studied samples were found to be very closely related to *K. pneumoniae* isolates. The sample (S9) was closely related in this phylogenetic tree to (MH930397.1, MK902672.1 *K. pneumoniae*, the closely related samples (S4, S6) by branch binding with MZ389287.1. The S1 sample was closely related to the reference sequence, as in Fig. 4; both samples were binding with adjacent branches with S3 samples, the reference sample and (S1, S3) binding to other samples that compared in this tree (MT052339.1,MT197277.1).

Table 1. sequences of primers used in gene amplification

Gene	Primer Sequences (5´- 3´)	Product size	Reference
16SrRNA	F*: AGAGTTTGATCCTGGCTCAG R*: GGTTACCTTGTTACGACTT	1500bp	[7]
K1	F: 5’AGATAGAGGTGTATTGTCGC R:GAGCTCTATATGTTGGATGC	352bp	[8]
K2	F: TCATACTTGACAGAGGGAGTAG R: ACGATCGTTACAGTGACAAG	321bp	[8]
blaSHV	F: GGCCGCGTAGGCATGATAGA R: CCCGGCGATTTGCTGATTTC	714bp	[9]

A: Adanin, T: Thaymin, C: Cytocin, G: Guanin

Table 2. The pattern of the observed differences between the NCBI reference sequences (GenBank accession number NZ_KQ088287.1) and the 16S rRNA amplicons' 1500 bp.

Sample	**Variant**	**Position in the PCR fragment**
S6	Tins34	34
S2,S4, S6,S7	C129T	129
S4,S6	T403G	403
S9	T403A	403
S2,S7,S9	G405A	405
S4,S6	G420A	420
S2,S7	T421G	421
S2,S7	Tins652	652
S2,S7	Ains653	653
S4	Tins770	770
S4	Cins771	771
S6	Gins835	835
S9	T960G	960
S6	A963G	963
S6	G964A	964
S9	G970C	970
S9	A971T	971

Figure 1. Agarose gel electrophoresis of 16SrRNA gene amplification, where M: ladder, 1-9: positive results.

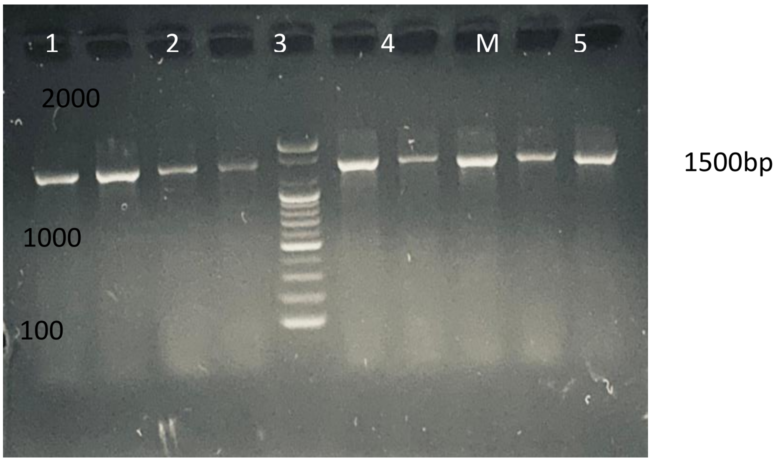


Figure 2. Agarose gel electrophoresis of *bla*$_{SHV}$ gene amplification, where M: ladder, 1-9: positive results.

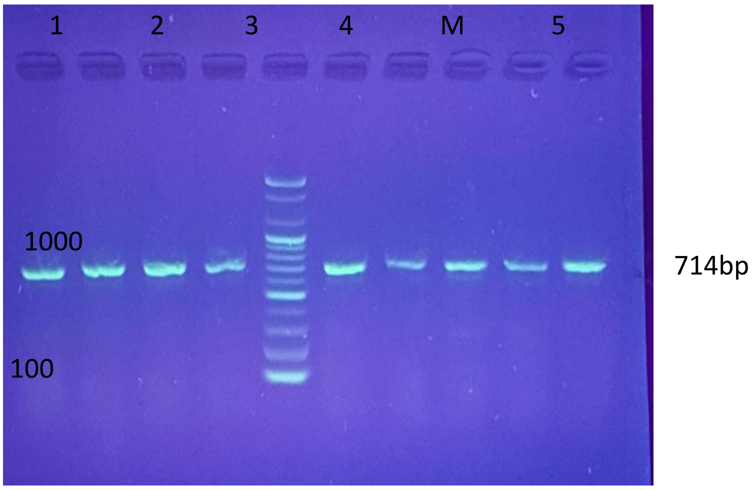


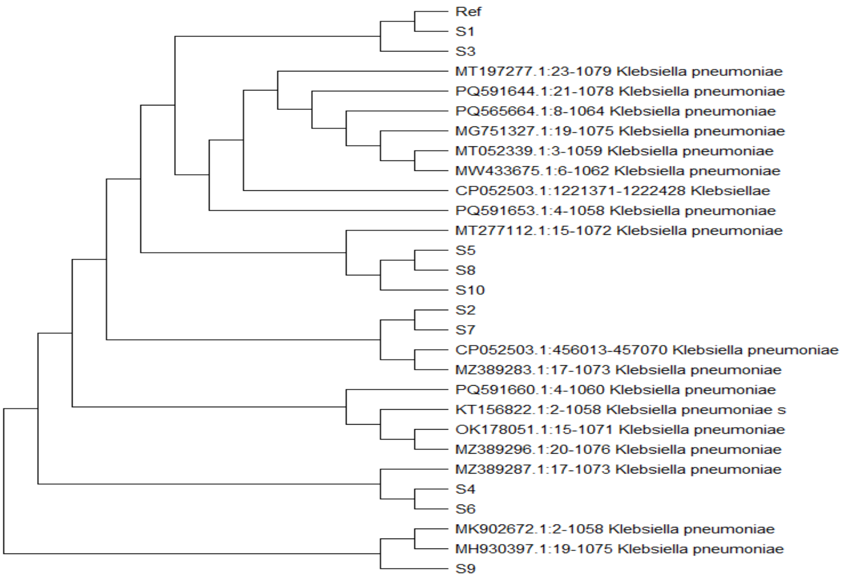
Figure 3. Ten bacterial samples' nucleic acid sequences were aligned with the reference 16S rRNA sequences found in the genomic DNA sequences of K. pneumoniae. "S" stands for sample numbers, while "ref" stands for the NCBI reference sequences.The precise locations of the detected alterations were detailed in Table 2 to provide a summary of all the findings derived from the 1500 bp segments that were sequenced

```
        90       100       110       120       130       140       150       160
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Ref  CCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGC
S1   ................................................................................
S2   ................................................T...............................
S3   ................................................................................
S4   ................................................T...............................
S5   ................................................................................
S6   ................................................T...............................
S7   ................................................T...............................
S8   ................................................................................
S9   ................................................................................
S10  ................................................................................
       410       420       430       440       450       460       470       480
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Ref  CGTTGAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT
S1   ................................................................................
S2   ....A...............G...........................................................
S3   ................................................................................
S4   ..G................A............................................................
S5   ................................................................................
S6   ..G................A............................................................
S7   ....A...............G...........................................................
S8   ................................................................................
S9   ..A.A...........................................................................
S10  ................................................................................
       650       660       670       680       690       700       710       720
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Ref  AATGCGTAGAG--ATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGT
S1   ...........--...................................................................
S2   ...........AT...................................................................
S3   ...........--...................................................................
S4   ...........--...................................................................
S5   ...........--...................................................................
S6   ...........--...................................................................
S7   ...........AT...................................................................
S8   ...........--...................................................................
S9   ...........--...................................................................
S10  ...........--...................................................................

       730       740       750       760       770       780       790       800
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Ref  GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG--TCGATTTGGAGGTTGTGCCCTTGAGGCGT
S1   .................................................--.............................
S2   .................................................--.............................
S3   .................................................--.............................
S4   .................................................TC.............................
S5   .................................................--.............................
S6   .................................................--.............................
S7   .................................................--.............................
S8   .................................................--.............................
S9   .................................................--.............................
S10  .................................................--.............................

       970       980       990      1000      1010
     ....|....|....|....|....|....|....|....|....|....|....|...
Ref  CCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCA
S1   ..........................................................
S2   ..........................................................
S3   ..........................................................
S4   ..........................................................
S5   ..........................................................
S6   ..GA......................................................
S7   ..........................................................
S8   ..........................................................
S9   ........CT................................................
S10  ..........................................................
```

Figure 4. The phylogenetic tree of the 16S rRNA gene in the *K. pneumoniae* samples under study is displayed. The heuristic search's initial tree or trees were automatically generated by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then choosing the topology with the superior log likelihood value. This analysis involved 29 nucleotide sequences, with the first, second, third, and noncoding codon positions included. The final dataset contained 1094 positions.



Discussion

The 16S rRNA gene sequences were used to identify all *Klebsiella pneumoniae* isolates obtained from various clinical sources, including blood, feces, urine, and sputum. These sequences were aligned and compared to reference strains deposited in the GenBank database. The results demonstrated that the 16S rRNA sequences from the present study were in agreement with findings reported by Budiarso [10].

It is important to note that the 16S rRNA gene contains hypervariable regions that vary among bacterial species. In this study, sequencing targeted selected hypervariable regions of the gene, which, although shorter in length, have been shown to provide reliable results comparable to full-genome analyses. However, it is worth noting that no single hypervariable region can effectively discriminate all bacterial species[11].

Several nucleotide polymorphisms were observed among the 16S rRNA sequences of the *K. pneumoniae* isolates. For instance, in samples S2 and S7, polymorphisms such as C129T, G405A, T421G, Tins652, and Ains653 were identified. Additionally, sample S9 exhibited unique mutations, including T403A, T960G, G970C, and A971T. Phylogenetic analysis revealed that isolates S2 and S7 were closely related.

This study is consistent with the finding [10].who identified 13 polymorphic nucleotide sites among *K. pneumoniae* strains. Notably, two isolates in the current study exhibited polymorphisms at positions 352 and 379 and showed substantial genetic distance from the other isolates. BLAST analysis indicated that some isolates shared high similarity with Micrococcus species, suggesting potential misidentification or a close genetic relationship with non-*Klebsiella* strains deposited in GenBank. Bacteria are inherently subject to mutations caused by environmental pressures. These genetic changes contribute to bacterial evolution and adaptation [1 2]. The similarity of K. pneumoniae isolates in this study with strains recorded in the NCBI database was confirmed through BLASTn analysis of the 16S rRNA gene, revealing up to 99.9% sequence homology with isolates from various countries (e.g., NZ_KQ088287.1,MH930397.1,MK902672.1).The observed genetic variation in the 16S rRNA gene of *K. pneumoniae* isolates from urine samples of pregnant women suggests a potential link to the source of infection. The phylogenetic proximity between human-derived and animal-associated strains highlights a possible zoonotic risk associated with contaminated food products [1 3]. The increasing prevalence of urinary tract infections caused by multidrug-resistant *K. pneumoniae* underscores the pathogen's enhanced virulence. This may be associated with nucleotide mutations that promote antimicrobial resistance and pathogenicity, highlighting the need for molecular surveillance [14].

Conclusion

Isolation of the *Klebsiella pneumoniae* causes urinary tract infection in pregnant women in Thi-Qar province and is detected molecularly. All *Klebsiella pneumoniae* isolates were confirmed as 100% *Klebsiella pneumoniae* due to 16S rRNA. *bla*SHV refers to rates of 100% in all *Klebsiella pneumoniae*. *Klebsiella pneumoniae* appeared with nucleotide maturation in 16SrRN, and ten isolates were recorded with NCBI. Finally, all *Klebsiella pneumoniae* appeared to have negative results for the K1 and K2 genes.

Acknowledgement

This paper is a part of our thesis (M.Sc.) which was carried out in the laboratories of Veterinary Medicine Colloge, University of Shatrah, Iraq.

Conflict of interest

None declared

Financial disclosure

None declared

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contribution

Nabaa Dakhil Httaihet conducted the laboratory work, data collection, and initial manuscript drafting. Haydar Khamis Almaliky: .The supervisor contributed to study design, data interpretation, critical revision, and final approval of the manuscript.

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