Original Article

Multilocus Sequence Typing of *Klebsiella* Species Strains from Conjunctivitis Causes in Children

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Abstract

Background: This study investigated the prevalence and distribution of *Klebsiella* species causing conjunctivitis in children at Mosul hospital. **Objectives:** The aim of this study was to examine the occurrence and distribution of *Klebsiella* species responsible for conjunctivitis in children admitted to Mosul Hospital. **Materials and Methods:** A total of 30 conjunctival samples were collected, from which seven Klebsiella strains were isolated. The strains were accurately identified using chromogenic agar, which provided distinct colors for precise identification. In addition, the VITEK-2 method was used to enhance visualization and aid in strain characterization. To assess genetic variation and classify the strains, multi-locus sequence typing (MLST) was performed. **Results:** Among the isolated strains, two were identified as *K. aerogenes*, whereas the remaining five were identified as *K. pneumoniae*. The application of chromogenic agar and the VITEK-2 method facilitated the identification and characterization of the strains effectively. MLST analysis revealed genetic relatedness and potential transmission routes within the hospital environment. Phylogenetic analysis showed varying genetic similarities among the *K. pneumoniae* strains. Notably, ST 448 and CAV 1344 clustered closely, suggesting a shared evolutionary history. ATCC 13884 showed unique genetic variations, whereas strain BWH2 showed close relatedness to other *K. pneumoniae* strains. Layla 1, a strain first recorded in Iraq, displayed distinct genetic characteristics. Among the *K. aerogenes* strains, ADL-323 and FGI35 clustered together due to shared genetic backgrounds. **Conclusion:** These findings contribute to our understanding of the population structure and evolution of *K. pneumoniae* and *K. aerogenes* strains causing conjunctivitis. The accurate identification of Klebsiella species is crucial for the development of effective treatment strategies and infection control measures. Molecular typing techniques play a significant role in investigating genetic diversity and epidemiology. Overall, this research sheds light on the specific Klebsiella species implicated in conjunctivitis among children at Mosul Hospital, emphasizing the necessity for further studies in this field.

Keywords: Children conjunctivitis, *Klebsiella* species, multilocus sequence typing, phylogenetic

Introduction

Conjunctivitis, commonly known as pink eye, is a prevalent eye infection that often affects children. It is characterized by inflammation of the conjunctiva, which is the thin transparent membrane covering the white part of the eye and the inner surface of the eyelids. Various bacteria have been identified as causative agents of conjunctivitis in children, including *Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus*, and *Moraxella catarrhalis*.[1] However, recent studies have also highlighted the involvement of gram-negative bacteria belonging to the *Klebsiella* species in conjunctivitis cases.[2-4]

Klebsiella is a classification category encompassing a diverse collection of organisms of microorganisms that fall under the category of gram-negative bacteria. These bacteria possess the characteristic of being facultative anaerobes, thereby enabling them to thrive and persist in environments that show both aerobic and anaerobic conditions.[5] The ecological distribution of *Klebsiella*

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species is notably extensive, with their presence observed in diverse niches such as soil, water, plants, and the gastrointestinal tracts of various animal species, including humans.[6]

Understanding the genetic diversity and relatedness of *Klebsiella* strains causing conjunctivitis is of significant importance for the development of effective diagnostic and treatment strategies. To achieve this, researchers have utilized a molecular typing method called multilocus sequence typing (MLST). MLST involves sequencing multiple housekeeping genes to determine the evolutionary relationships between bacterial strains.[7,8] This approach has proven valuable in exploring the diversity and epidemiology of *Klebsiella* species.[9]

Chromogenic agar represents an advanced and sophisticated diagnostic tool utilized for the expeditious and accurate identification of *Klebsiella* species. This specialized agar medium incorporates chromogenic substrates that undergo discernible color transformations in response to the selective enzymatic activity shown by *Klebsiella*. This innovative technology allows for the expeditious differentiation of *Klebsiella* from other bacterial counterparts, significantly reducing the time required for identification. Notably, the presence of the enzyme β-galactosidase, which is produced by *Klebsiella pneumoniae*, elicits the development of distinctive blue-hued colonies on the chromogenic agar medium. By harnessing this efficient and precise method, healthcare professionals can promptly detect and characterize *Klebsiella* species, thereby enabling timely diagnosis and efficacious management of infections attributed to these bacteria.[10]

The goal of the study detailed in the provided sources is to utilize MLST for the purpose of characterizing *Klebsiella* strains associated with conjunctivitis in children. By doing so, the researchers aim to gain insights into the epidemiology and pathogenesis of *Klebsiella*-associated conjunctivitis and provide essential information for the development of diagnostic and therapeutic approaches. This research builds upon existing knowledge of conjunctivitis-causing bacteria and aims to enhance our understanding of this common eye infection in pediatric populations.

Materials and Methods

A total of 30 samples were obtained from children aged 1 month to 6 years diagnosed with conjunctivitis at Mosul hospitals between January 2021 and December 2022. The identification of these isolates was performed utilizing a novel technique that combines the utilization of Chromogenic agar (CHROMagar KPC) and the Vitek 2 system, along with standard biochemical tests. The utilization of Chromogenic agar, a specialized growth medium, facilitated the distinction and recognition of different *Klebsiella* species by leveraging their distinct enzymatic activity. Concurrently, the Vitek 2 system augmented the identification process through the implementation of biochemical tests. This integrated approach effectively led to the successful identification of *Klebsiella* isolates, enabling precise classification and characterization of the strains implicated in conjunctivitis cases among children.[10] In order to ascertain the sequence types (STs) of the *Klebsiella* strains, Multilocus sequence typing (MLST) was conducted.[11,12] This involved the amplification and sequencing of seven housekeeping genes, namely *gapA, infB, mdh, pgi, phoE, rpoB, and tonB* in terms of *K. pneumoniae* [Table 1], whereas in *K. aerogenes* there are another housekeeping genes included *dnaA,fusA,gyrB, leuS, pryG, rplB and rpoB* [Table 2], through the utilization of PCR.[13] The resulting sequences were subsequently subjected to analysis using the MLST database, which facilitated the identification and classification of the strains based on their unique sequence profiles. The MEGA program was used to construct a phylogenetic tree using MLST sequencing data for analysis. By using this methodology, comprehensive insights into the genetic diversity and relatedness of the *Klebsiella* strains were attained, allowing for a more robust understanding of their epidemiology and pathogenesis in the context of conjunctivitis cases.

A phylogenetic tree was constructed using the concatenated sequences of the seven housekeeping genes for all *Klebsiella* strains. The tree was generated using the maximum likelihood method in MEGA X software.[3]

The data were analyzed using SPSS software version 26.0. The chi-square test was used to compare the distribution of antibiotic resistance among different STs.[4]

Table 1: MLST oligonucleotide sequences (5′–3′) for *K. pneumoniae* primers for this study

Name of gene	Forward primer 5–3	Reverse primer 5–3	Length (bp)
gapA	ATGGCGGCAATGGTTCAA	TTACGCGCACCTTGACCTG	460
infB	TGAGCGATGCTGATGTTG	CCGCGACAGACAGATCG	319
mdh	GGGAGTTGATGCTGACGTTG-	CCGCTTTGATGCTGAGTTC	480
pgi	TTGGCGTCTGCTGAATGAC	GGGCGTGTGATGAAGTTG	434
phoE	CGAACTACGGTGAACGGT	TCCCGGCTTATCGCATTG	421
rpoB	TGCATCATCCTGAACCGG	CGCGTTGTCGTTGCTGTT	504
tonB	GCCGATCGCTGCTGAAGT	CCGGCGATGTTGCTGTAT	432

Table 2: MLST oligonucleotide sequences (5′–3′) for *K. aerogenes* primers for this study

Name of gene	Forward primer 5′–3′	Reverse primer 5′–3′	Length (bp)
dnaA	CGTAAGCCAAATGCTAAAGTG	GGCAGGCTGTGGTTGGTC	442
fusA	GTTCTTGATGGTGCGGTAATG	CATACGCTCCAGAATGATCG	646
gyrB	GCGACGGCAAAGAAGACC	CCTGGTTCTTACGGTTACG	434
leuS	CAATTCCAATGGTGACCCTG	CGGTATTGAAGGTCTGACG	578
pryG	CGTGACCGTCAACATCAAGC	CTTCAATCGGTTTCAACAACA	263
rplB	ATGGCAGTTGTTAAATGTAAAC	TCAGTACGCTTGTTGCTGC	607
rpoB	GCTACCGTCTGGGCGAAC	TCGTTGGTGAAGAGCGTTTC	545

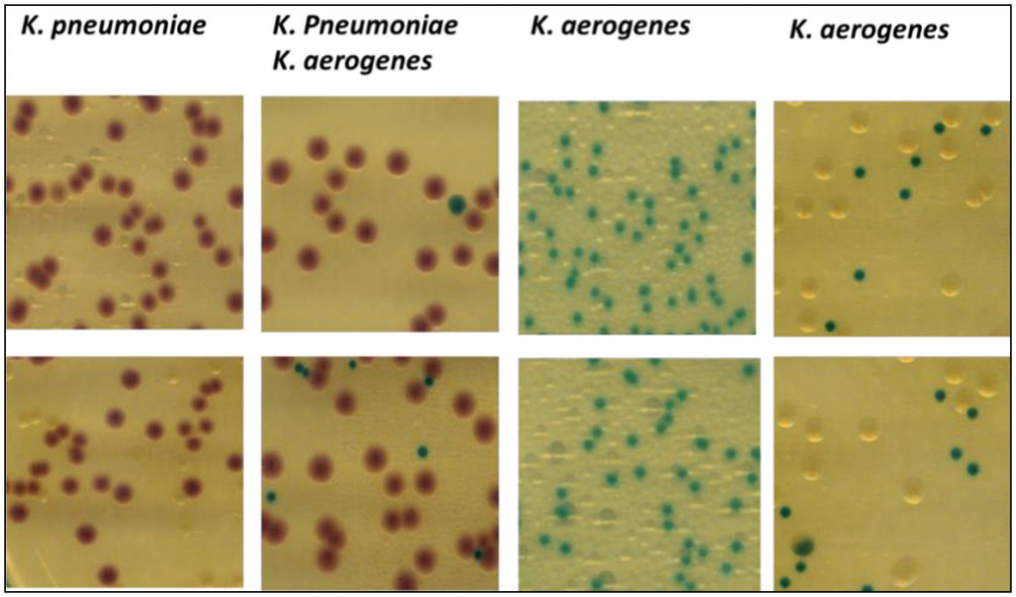

Figure 1: Chromogenic agar enables accurate species-level two strains identified as *K. aerogenes* green color and five strains identified as *K. pneumoniae* (red–pale color), identification of *Klebsiella* strains associated with conjunctivitis

Ethical approval

This study, documented under reference number [4S/33], has received ethical approval from the Scientific Committee in the Department of Medical Physics/ University of Mosul, during the thirteenth session held on the 13th of March, 2023.

Results

The study examined a total of 30 samples collected from children with conjunctivitis at Mosul Hospital. Among these samples, only seven strains of *Klebsiella* species were successfully isolated and utilized for further analysis. The strains were identified using chromogenic agar, and their visualization was enhanced using the VITEK-2 method. Additionally, MLST was used to obtain a more comprehensive understanding of the strains. The results indicated that two of the isolated strains were identified as *K. aerogenes*, whereas the remaining five strains were identified as *K. pneumoniae.*

This research provides important insights into the prevalence and distribution of *Klebsiella* species causing conjunctivitis in children at Mosul Hospital. The use of chromogenic agar, as shown in Figure 1, facilitated accurate identification of the strains based on their distinct colors. Furthermore, the application of the VITEK-2 method, The display improved strain visualization, aiding in their characterization [Table 3].

The utilization of MLST, as presented in Figure 2 and 3, played a crucial role in classifying the isolated strains, allowing for the evaluation of genetic variation among different isolates. This information is essential for understanding the relatedness and potential transmission routes of *Klebsiella* strains within the hospital environment. Furthermore, this understanding holds the key to comprehending how these strains could potentially move through the hospital environment, potentially infecting individuals and necessitating targeted preventive strategies. In essence, MLST serves as a powerful tool that empowers researchers and healthcare professionals alike to proactively manage and mitigate the spread of Klebsiella infections within the hospital context.

Klebsiella pneumoniae strains show varying degrees of genetic similarity and divergence. ST 448 and CAV

Table 3: VITEK 2 test for *Klebsiella* species: Identification number, test name, and reaction (+/−/Ind) indicating biochemical activity

ID	*Klebsiella* species/biochemical test—VITEK 2	Results
1	GN-ID	+ve
2	GN-CARD	+ve
3	GN-CARD (AST)	+ve
4	GN-CARD (ID)	+ve
5	GN-CARD (MIC)	+ve
6	Extended-spectrum beta-lactamase (ESBL) confirmatory test	+ve
7	Carbapenemase test	−ve
8	ESBL/AmpC gene detection	+ve
9	KPC gene detection	−ve
10	OXA-48 gene detection	−ve
11	AmpC gene detection	−ve
12	Metallo-beta-lactamase gene detection	−ve
13	ESBL	+ve

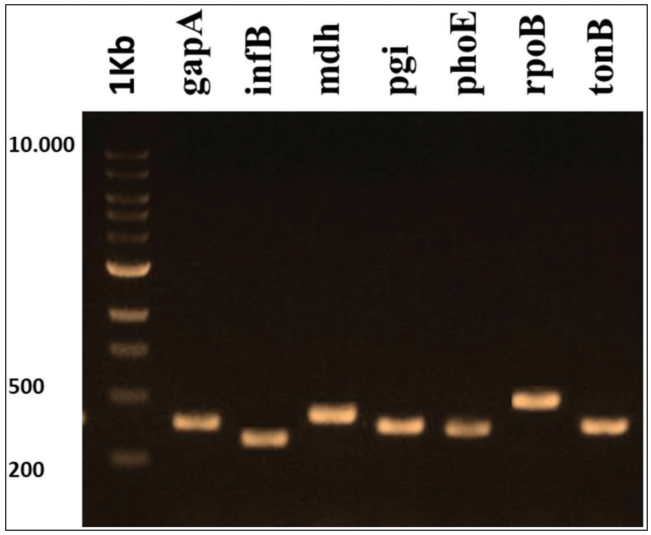

Figure 2: Distinct bands signify genetic variability among *K. pneumoniae* strains in the PCR gel

1344 cluster closely with other *K. pneumoniae* strains, indicating a shared evolutionary history. ATCC 13884 forms a distinct cluster, suggesting unique genetic variations. BWH2 shows close relationship with other *K. pneumoniae* strains. Layla 1, a strain first recorded in Iraq, branches separately, potentially indicating distinct genetic characteristics. For *K. aerogenes* strains, both ADL-323 and FGI35 cluster closely with other *K. aerogenes* strains, implying a shared genetic background [Figure 4].

The phylogenetic tree analysis highlights the evolutionary relationships and genetic relatedness among the strains, providing valuable insights into their diversity and potential origins. These findings contribute to our understanding of the population structure and evolutionary dynamics of *K. pneumoniae and K. aerogenes.*

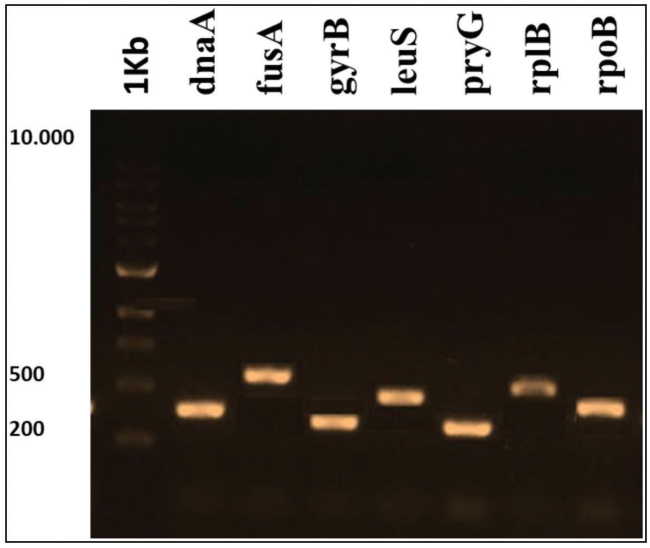

Figure 3: Distinct bands signify genetic variability among *K. aerogenes* strains in the PCR gel

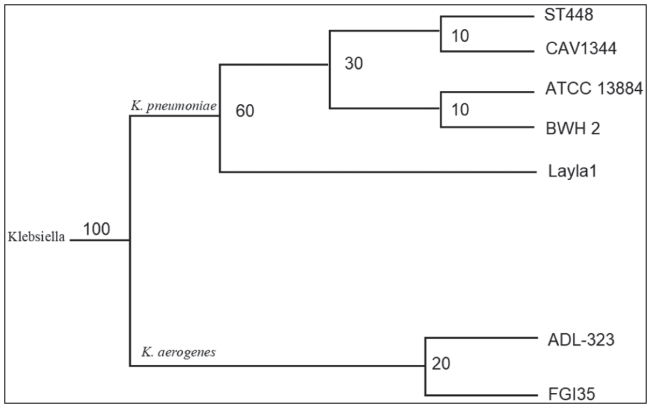

Figure 4: Phylogenetic tree depicting the evolutionary relationships among *Klebsiella pneumoniae* and *Klebsiella aerogenes* strains based on MLST sequencing

The identification of two strains as *K. aerogenes* and five strains as *K. pneumoniae* underscores the significance of accurate species-level identification for appropriate treatment strategies and infection control measures. These findings emphasize the importance of using molecular typing techniques, as highlighted to investigate the genetic diversity and epidemiology of *Klebsiella* strains associated with conjunctivitis.

Overall, this study provides valuable information regarding the specific *Klebsiella* species involved in conjunctivitis among children at Mosul Hospital. The results highlight the importance of using various methods for identification and characterization and emphasize the need for further research in this area.

Discussion

The study's objective, as outlined in the referenced sources, is to use MLST for characterizing *Klebsiella* strains linked

to conjunctivitis in pediatric cases.[3,14] A total of thirty samples were collected, from which seven *Klebsiella* strains were isolated and analyzed. The strains were identified using chromogenic agar, which facilitated accurate identification based on distinct colors.[15,16] The VITEK-2 method was used to enhance strain visualization and aid in their characterization.[17,18]

To gain a comprehensive understanding of the strains, MLST (Multi-Locus Sequence Typing) was utilized.[19] The MLST analysis revealed that two of the isolated strains were identified as *K. aerogenes*, whereas the remaining five strains were identified as *K. pneumoniae*.[19-21] The obtained MLST data were further analyzed through phylogenetic tree construction using the MEGA program.[22,23] The phylogenetic tree highlighted the genetic relationships among the strains, providing insights into their diversity and potential origins.[24]

The analysis of *K. pneumoniae* strains showed varying degrees of genetic similarity and divergence. Strains such as ST 448 and CAV 1344 clustered closely with other *K. pneumoniae* strains, indicating a shared evolutionary history.[25] ATCC 13884 formed a distinct cluster, suggesting unique genetic variations, whereas BWH2 showed a close relationship with other *K. pneumoniae* strains. Layla 1, a strain first recorded in Iraq, showed distinct genetic characteristics, branching separately. Regarding *Klebsiella aerogenes* strains, both under-clustered closely with other *K. aerogenes* strains, indicating a shared genetic background.[26]

The findings of this study provide valuable information on the prevalence and distribution of *Klebsiella* species causing conjunctivitis in children at Mosul Hospital.[14] Accurate species-level identification, as achieved through the used methods, is crucial for appropriate treatment strategies and infection control measures.[27-29] The utilization of MLST and phylogenetic tree analysis contributed to the understanding of genetic variation, relatedness, and potential transmission routes of *Klebsiella* strains within the hospital environment.[30-32]

This study has certain limitations, including the small sample size and the focus on a specific hospital setting. Therefore, further research with larger sample sizes and inclusion of multiple healthcare facilities is warranted to gain a comprehensive understanding of the prevalence and genetic diversity of *Klebsiella* species causing conjunctivitis.[33]

In summary, this study sheds light on the specific *Klebsiella* species involved in conjunctivitis among children at Mosul Hospital. The used identification and characterization methods, along with molecular typing techniques, provide insights into the diversity and epidemiology of *Klebsiella* strains.[34-36] Further research in this area is necessary to advance our knowledge and develop effective strategies for prevention and treatment.[37]

Conclusion

In conclusion, this study investigated the prevalence and distribution of *Klebsiella* species causing conjunctivitis in children at Mosul Hospital. Seven strains were isolated, including the first-time recorded strain named Layla 1, which represents the first isolation of its kind in Iraq. The findings contribute to our understanding of the genetic diversity and epidemiology of *Klebsiella* species associated with conjunctivitis, highlighting the importance of accurate species identification for effective treatment and infection control measures.

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Conflicts of interest

There are no conflicts of interest.

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